

Margaret B. Scheer

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ALBERT JAN KLUYVER

HIS LIFE AND WORK



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ALBERT JAN KLUYVER

HIS LIFE AND WORK

BIOGRAPHICAL MEMORANDA

SELECTED PAPERS

BIBLIOGRAPHY AND ADDENDA



1959

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PREAMBLE

THIS book has been conceived in consultation with the children of the late Professor Kluyver. Colleagues, pupils, collaborators, and other associates from well-nigh every period of his life have unstintingly responded to requests for contributions to the biographical section. The result aims at bearing witness to a sense of solidarity and reverence which is also manifested, as replica in effigy, by the bust sculptured by Professor Wenckebach through the initiative of Kluyver's pupils. This bust was installed in the new Laboratory of Microbiology when the latter was being occupied; at that time, too, the plaque of Beijerinck was moved thence from the now abandoned old laboratory at the Nieuwelaan where the two scientists had conducted their illustrious studies for so many decennia.

A strong impetus to the publication of the 'Selected Papers' was the desire to render comprehensible to future generations the influence that Kluyver's intellectual and human traits have exerted on science and on his associates in the widest sense of the word.

Referring to the Apology for more specific details, a brief account of the composition of this book is here presented.

Initially the possibility was considered of reprinting all of Kluyver's publications. Closer scrutiny made it necessary to abandon such a plan. In part this was motivated by the fact that many of the concepts enunciated by Kluyver have long since been firmly incorporated into microbiological science. Others, representing notable stages in the development of this discipline at the time of their publication, have lost some of their significance in the course of years on account of later improvements in methodology and understanding. The scales were turned by the decision to let Kluyver's work and personality speak to as large an audience as possible; and this inevitably implied a selection.

Had this been confined to a choice from among the strictly original scientific papers, the resulting collection would have given too one-sided a picture of Kluyver's range of activities. Consequently a num-

ber of review articles and the text of some lectures have been included; for particularly in these lectures he displayed that harmonious mastery over form and content, that highly developed linguistic and vividly stylistic ability that were so characteristically his own. The essence of this style reveals itself not only when he wrote in his mother tongue, but it is equally apparent from his publications in English, French, and German. At least one paper in each of these four languages has been incorporated here, while several papers, hitherto available only in the Dutch language, have been inserted in English translations. In this manner the collection may offer some novelties to interested people in foreign countries.

It stands to reason that ample space has been reserved for an essay on Kluyver as a scientist, in which an attempt has been made to assess the totality of his contributions.

The biographical section was written by several authors, each one covering a particular period of his life. For its composition contributions were received from a former secondary school classmate who has written about this phase; from a colleague whom Kluyver befriended when they both were first-year students at the University, and who has dealt with the years prior to Kluyver's assumption of the professorate; and from all those who were consecutively associated with him as senior staff members during the years of his professorship, and have covered this period.

The biographical section closes with two orations spoken at the cremation ceremonies at Westerveld, *viz.*, the one delivered by the Rector Magnificus of the Technological University in the name of organizations and persons within the confines of the University, and the testimonial of Kluyver's closest friend, especially rewritten for publication in this book.

The bibliography, finally, lists all of Kluyver's publications. In addition it contains a record of the Doctor's dissertations and papers issued from his laboratory that do not bear his name. Apparently the Director did not have a rigid rule for determining whether or not his name should appear on a publication. Even so, the work done by his pupils was often concerned with the elaboration and testing of ideas that he supplied; and in spite of a consummate respect for the individuality of his coworkers he was wont to instill into every paper from his institute a characteristic and highly personal flavour.

Although the entire book is a memorial to Kluyver, it seemed warranted to include a single 'In Memoriam'. The choice fell on the one that appeared in the 'Nieuwe Rotterdamse Courant' of May 15, 1956, written under the immediate influence of the realization of the loss that had been suffered. The present volume contains moreover a list of the numerous grateful appreciations written by Dutch and foreign scientists.

Thus goes out into the world, and for the last time, a creation of A. J. Kluyver that represents an impressive part of his efforts; yet for the first time it has not been supervised by his own meticulous care. The compilers have ever been conscious of this regretted circumstance, and they have recognised how greatly it has added to their responsibility.

May the reader, even of a future generation, glean from this volume some of the benefits that the lustre of Kluyver's mind and personality so generously bestowed upon all who knew him.

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Chemie der Zelle und Gewebe 13, 134, 1926.

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Annales de Zymologie Série II, 50, 48, 1931.

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Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten.

Abt. II, 94, 369, 1936.

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Translation of: 'Microbe en Leven'.

Jaarboek der Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam, 1954-1955, p. 225.

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PART ONE

BIOGRAPHICAL MEMORANDA

FROM YOUTH TO PROFESSOR

THE EARLY YEARS

1888-1905

ALBERT JAN was the second child and only son sprung from the marriage of Jan Cornelis Kluyver and Marie Honigh. His father, born May 2, 1860, in Koog aan de Zaan, came from a family of merchants who for many generations had been living in the Zaan district, a region in the vicinity of Amsterdam. The family name is associated with ships; a 'kluiver' is a jib. Jan Cornelis and his somewhat elder brother, Albert, both were outstanding students at the but recently established secondary school in Zaandam, and the first members of the family to continue their formal education after graduating from this school. Albert chose the study of Letters at Leiden University, became editor of the Netherlands' Dictionary, and afterwards professor at the University of Groningen. Jan Cornelis attended the Polytechnical College in Delft where he received the engineering degree, and at the age of 32 became professor of mathematics in Leiden. Thus the studies of these two brothers have notably enriched Dutch scholarship.

The first position occupied by Jan Cornelis was that of instructor in mathematics at the Governmental High School in Breda (1883). During his first year there he married Marie Honigh, likewise from the Zaan district, and who at the time was teaching in Haarlem (born September 27, 1859, in Zaandijk). In Breda a daughter was born to them, and a few years later, on Sunday, June 3, 1888, the son, Albert Jan. In 1890 the family moved to Amsterdam, where Kluyver had been appointed to the faculty of the Municipal High School at the Weteringschans. Thence he was called to the professorate at Leiden University, in 1892. Both in Amsterdam and in Leiden the family was augmented by the birth of a daughter. In 1896 the University of Groningen conferred upon Jan Cornelis Kluyver an honorary doctorate.

In Leiden the family first occupied a none too cheerful house in the Rembrandtstraat, though it commanded from the rear a magnificent

view of the Witte Singel, a local canal. Later they moved to an architecturally interesting, old-fashioned house on the Heerengracht. Finally, to the delight of father Kluyver, the family acquired a very modern, sunny, and colourful mansion at the Hoge Rijndijk; it was from here that the children gradually dispersed.

Kluyver died in Leiden, at the age of 72, on December 3, 1932, four years to a day after his wife. He was an exceptionally thorough mathematician, imbued with the contributions of the great mathematicians of the past, from whose works he often borrowed illustrative examples. When lecturing he was wont to cover blackboard after blackboard with beautifully regular letters and signs, without ever wavering. He was a stately, imposing figure, with a wittily ironic gleam in his grey eyes that caused many students rather to fear him. He was a splendid speaker and the address on 'The Steady Evolution of Mathematics' which, as Rector Magnificus, he delivered on February 8, 1910, in the main auditorium of Leiden University, remains in my opinion the most beautiful paper on mathematics for laymen.

It was after the Easter holidays in 1904 that I first set eyes on Albert Jan. He occupied a front row seat in the fourth grade classroom of the Leiden High School; a slender, blond boy, but recently graduated to 'long pants'. Invariably he knew everything, and never failed a quiz; but he was in no way a braggart, rather did he appear timid and bashful.

Not till after the summer holidays, when we had frankly expressed our delight at meeting again, did we begin regularly to study together for the final examinations. Mostly we worked in his room which we had virtually converted into a laboratory for practicing chemistry. Nearly every evening was concluded by a brief visit to the living room. Like all living-rooms in those days, this was a simply furnished apartment, with a rectangular table in the centre over which hung the lamp; there were no low little tables or cosy nooks. During the winter Albert Jan did his homework at that table in the family circle; his powers of concentration made him immune to distraction. On rare occasions, when upstairs we got stuck in a mathematical problem, we mustered up enough courage to ask the professor how it should be tackled. Usually he was engaged in reading a book or newspaper, and, almost without looking up or listening to us, he would then sketch

the main elements of the solution in the margin of the paper, while making no bones about showing that he thought us far from bright. The presence of Mrs. Kluyver created in that room an atmosphere of order and kindness that has remained with me to this day, half a century later.

The final examinations became a great success. In those days they were not handled in and by the school, but administered by a large board which, in 1905, met in the city of Gouda. At the time this place could be reached from Leiden only by a combined railway and horse-drawn tramcar voyage. The trip to, and the sojourn in Gouda were a very special event for the three of us, the third one being C. Punt, who later became known as Kluyver's partner in the famous tennis double.

Outwardly Albert Jan was the image of his father, distinguished-looking, though a little less formal; and from his father he probably inherited also his uncommonly sharp intellect. But the charm that he radiated and that affected every one he met; his capacity for ingeniously paying close attention to every human being; these he owed to his mother. And that was not the smaller part of the gift of grace that had been granted him in the form of his excellent parents.

W. E. v. W.

STUDENT AND ASSISTANT

1905-1916

In September, 1905, Kluyver registered as a first-year chemistry student at the Technological University in Delft. Our first meeting occurred during the hazing days, in the apartment of P. J. van Voorst Vader, an older student, living at Oude Delft 128a. J. P. Valkema Blouw, another older student, was at the piano singing Speenhoff ditties which were much in vogue at the time. A few freshmen, among them Kluyver and I, had been herded under the table; here, in somewhat tenebrous circumstances and crouching position, began our acquaintance that was to lead to a life-long friendship.

From 1905 till 1907 Kluyver resided in Delft; I noticed comparatively little of him. After his sophomore year he moved to Leiden where for some years he roomed in the 'Pancras House', Hooglandse Kerkgracht 21, together with three students at Leiden University who studied chemistry, medicine, and theology, respectively.

During those years Kluyver, though a member of the Delft student

fraternity, did not care much for that city. To be sure, he had decided to become a chemical engineer; but his heart was set on Leiden from where he commuted to Delft. Here he participated but little in the activities of the student community; and, after the day's work was done, he always returned to Leiden where, apart from rooming with his student friends, he also took part in community affairs. Having grown up in that city he knew many local families, and was a member of the lawn-tennis club 'Ready' and of other social clubs.

In 1909 he passed, 'with distinction', the examinations that admitted him to candidacy for the degree of Chem. E. During his final year he studied mostly under Dr. J. Böeseken, Professor of Organic Chemistry, and in June, 1910, he received the Chem. E. degree, again 'with distinction'.

Meanwhile he had taken several courses under Dr. G. van Iterson Jr., Professor of Microscopical Anatomy, who had offered him, even before the final examinations, a position as assistant in his institute.*

It was during this period that a number of chemistry students, Kluyver among them, founded the 'Natural Sciences Dispute' which later became the nucleus of the Delft Section of the Netherlands' Chemical Society. At its monthly meetings one of the members discussed a subject in the natural sciences. Kluyver chose as his topic the chemical structure of chlorophyll. This lecture was published in 1911 in the 'Pharmaceutisch Weekblad' under the title, 'The chemistry of chlorophyll in the light of recent investigations'. It was Kluyver's first publication, and he was not a little proud of it. But at the same time he realized even then the relativity of all things. The reprint he presented to me had been inscribed in his fine, sharp script with the following quotation from Otto Ernst's 'Semper der Jüngling', a widely read novel at that time: 'Zwar blieb seine Wissenschaft einigermassen an der Oberfläche; er sprach allerlei vom Chlorophyll, aber was es für eine Bedeutung hatte wusste er eigentlich selbst nicht'.

During his student years Kluyver showed himself to be possessed of great mental ability, strong interests, and an extreme sense of duty. But it must not be concluded that he did nothing but study; in Leiden he cultivated that social intercourse with friends of both sexes which he needed so much. Here, especially during vacations, he indulged in

* At the Technological University this position carried various major responsibilities, and was generally occupied only by persons with an advanced degree.

sports; hockey in winter, and tennis during the remainder of the year. In tennis he excelled, and in those days he was one of the strongest players in Holland. The crowning glory came when, around 1910, he was a member of the Dutch team that was to play against a Belgian one, and won both singles matches. Soon thereafter he put his racket away, however, so that he could devote himself exclusively to his studies!

From 1910-1916 Kluyver was assistant at the Laboratory for Microscopical Anatomy, later renamed Laboratory for Technical Botany. It was a period during which not many industrial positions were open. Moreover, Kluyver wanted to spend at least some years in pure research; and in Van Iterson's institute he had a splendid opportunity to acquaint himself with various aspects of botany and eventually to master the field. The first two years offered scant opportunity for investigations of his own. He assisted with everything pertaining to the teaching functions of the laboratory, such as the preparation of lecture demonstrations and of the laboratory courses. This involved a great deal of work, particularly because the institute's staff could boast only one technician and a single gardener. Furthermore, he assisted in the laboratory courses, and partly supervised the work of the students specializing in the field. And when, in the spring of 1911, Van Iterson travelled to the Dutch East Indies as a delegate of the Netherlands' Government to the International Fibre Congress in Surabaja, Kluyver was left in complete charge of the laboratory for several months.

During the summer vacation of 1911 he spent some months working under Prof. Dr. Hans Molisch at the Plant-Physiological Institute of the University of Vienna. This led to a paper entitled 'Observations on the effect of ultraviolet radiation on higher plants' [1911]. He relished the beauties of Vienna, and returned home via the Dolomites.

It was logical to expect that Kluyver would attempt to prepare a Doctor's dissertation. In the spring of 1912 he began an investigation of biochemical sugar determinations, with the aim of elaborating this subject and, if possible, using the results for a thesis. For more than two years Kluyver threw himself into the problem with all his energy and endurance. Meanwhile he had moved back to Delft so that he could spend all his time on this study. This was particularly necessary because he regularly had to work evenings in the laboratory, and

often the observations had to be extended till deep into the night. The preliminary results of his investigations were reported at the 14th 'Netherlands' Congress of Natural Sciences and Medicine', in Delft, 1913.

On May 15, 1914, Albert Jan Kluyver received the degree of Doctor of Technical Science 'with distinction' on the basis of a sterling dissertation, 'Biochemical Sugar Determinations', accompanied by no less than 27 propositions.

It was just in time; on August 1 of that year the Dutch army was mobilized following the beginning of the first world war. Kluyver, too, was inducted into the army as buck private in the 4th Infantry Regiment in Leiden. He was fortunate in that his regiment was stationed there during the winter of 1914, so that he could spend his evenings with family and friends. His military career was not spectacular; he did not get promoted beyond the rank of corporal. But this was a blessing because it meant that, owing to Van Iterson's urgent remonstrances that Kluyver could not be spared at the institute, he could get a discharge in May, 1915. Thus Kluyver returned to Delft and his assistantship.

He was now 27 years old, and it became time to think of a future career. Van Iterson realized even then that this young man with his brilliant intellect would be capable of accomplishing great things. He considered it important that before settling down to a position in Holland, Kluyver should acquaint himself with life in the Dutch East Indies, or, more generally, in the tropics. The opportunity offered itself rather unexpectedly; at the Department of Agriculture, Industry, and Commerce in Buitenzorg a post had been created for a consultant whose duty it would be to promote the native industries. Dr. H. J. Lovink, Director of Agriculture in the above-mentioned Department, consulted Van Iterson, and subsequently interviewed Kluyver for this position. The latter accepted, partly on Van Iterson's advice.

Meanwhile another and most fortunate change had taken place in Kluyver's life. He had become engaged to Eja van Lutsenburg Maas, candidate for the Chem. E. degree, who was a student at the Laboratory for Technical Botany. This event undoubtedly influenced Kluyver's decision to accept the consultantship in Buitenzorg; now he knew that he need not feel lonely there.

On July 29, 1916, Albert Jan Kluyver and Helena Johanna van

Lutsenburg Maas were married in The Hague. Following a brief honeymoon in Holland – it was war time – they embarked early in August for a much longer voyage. During those war years the trip East was not so simple and took a very long time. England forced the Dutch steamers to sail around Scotland, and to dock at both Kirkwall and Falmouth for a careful scrutiny of passengers and cargo. Subsequently the ships sailed, via the Canary Islands and Capetown, around Africa. This provided an opportunity to visit his brother-in-law, Pijper, who was then a physician in South Africa, and later became professor of Bacteriology. The trip took more than two months, and not until October 10 did the Kluysers land in Tandjong Priok. After a brief sojourn in Batavia they continued on to Buitenzorg, the residence of the new industrial consultant.

THE TROPICAL PERIOD

1916–1921

Kluyver spent three wonderful years (1916–1919) in Buitenzorg. His numerous letters written during that time give a clear picture of this period that was of such importance to him. He learned much from his contacts with various functionaries, both in his department and in other institutions; he became familiar with the bureaucratic and political atmosphere in Buitenzorg which, to be sure, disappointed him in many respects, but nevertheless increased his worldly wisdom. He was glad not to have missed this experience.

He was disappointed with his field of activities, because he found neither a concrete plan, nor guidance as to definite lines of action. There was uncertainty and conflict. Initially, the Netherlands' Government had intended that the consultant-to-be should devote himself to the study, promotion, and development of native industries. This was opposed by those who wanted to involve the consultant in the promotion of European enterprises. Throughout the years of Kluyver's civil service (1916–1919) it thus remained in doubt for whom he should work, and the young consultant received too little support from his superiors in his difficulties. Furthermore, such plans as the creation of an independent department of industry, with an adequate laboratory in Bandung, did not materialize; the responsible officials had neglected to place the necessary funds on the 1919 budget.

Was it surprising that, with the approaching termination of his first term of office, Kluyver was doubtful of his future? An attractive prospect in the Indies was the above-mentioned separate department in Bandung, perhaps under his own direction. But it appeared that he had not been forgotten in Holland. The Colonial Institute in Amsterdam offered him the directorship of a department for scientific research that was soon to be created and would be charged with the study of problems in colonial technology. In conjunction with this position, Van Iterson wanted to attach him also to the Technological University in Delft as extra-ordinary professor of phytochemistry. And when Van Iterson cabled him that the Government had agreed to the creation of such a chair, and that the Delft faculty concurred with Van Iterson's nomination of its future occupant, Kluyver accepted the position at the Colonial Institute.

His first task was to make a survey of the coconut-fibre and copra-yarn industry in Ceylon, and on the Malabar Coast, with a view to determining whether such industries could be developed in Java.

However, just when Kluyver's European future seemed assured, but before starting his work with the Colonial Institute, an entirely different possibility opened up. This was a position with the Oil Manufacturing Company 'Insulinde'. Up to that time vegetable oils had been produced by purely empirical methods. The new director, M. H. Damme, Mech. E., recognized nevertheless that, were the industry to remain flourishing, scientific research would be imperative. Repeatedly he urged Kluyver to join this concern as chemical adviser, in which position he would become director of a new, well-equipped laboratory in Bandung, and under exceptionally favourable financial conditions. The offer was so enticing that Kluyver finally accepted, after the Colonial Institute had been found willing to cancel the previous arrangement, though with the stipulation that the survey of the copra-fibre industry would still be undertaken.

During the Buitenzorg episode the Kluyvers had made friends with several families, such as the Ruttens; Dr. L. Rutten was a geologist in the employ of the B.P.M., and later became professor in Utrecht. Others were Dr. Otto de Vries, Director of the Central Rubber Research Station, and subsequently extra-ordinary professor at the Medical University in Batavia; and H. J. Hellendoorn, Chem. E., also associated with the Rubber Research Station.

Kluyver terminated his work at the Department of Agriculture, Industry, and Commerce, and moved from his residence in Buitenzorg, Tjikeumeuh 32, where the couple had spent three happy years. Mrs. Kluyver traveled with their year-and-a-half old daughter to Holland, there to await the birth of her second child.

For the study of the copra-fibre industry Kluyver, accompanied by Raden Mas Iso Reksohadiprodjo, Agric. E., trained in Wageningen, and an instructor in agriculture in Java, embarked early in December, 1919, on the S.S. 'Nawab'. They first went to Ceylon, thence to the Malabar Coast, via Madras to Calicut, and later to Cochin (Travancore). Via Medan they returned to Java for consultation with the appropriate authorities and a discussion of the prospects for a copra industry. In May, 1920, Kluyver went to Holland in order to deliver a preliminary oral report at the Colonial Institute. The printed report of this enterprise appeared in 1923 as Communication No. XX of the Colonial Institute under the title, 'Copra-fibre and Copra-yarn Industry', a book of 300 pages, illustrated with beautiful photographs taken by Kluyver himself, and supplemented with numerous tables, maps, and appendices. It must be considered as the standard work on this difficult subject.

Not till November, 1920, did Kluyver return to Java with his wife and children – in Holland a son had been added. He installed himself in Bandung, where the family enjoyed the friendship of Dr. J. Clay, professor at the local Technological University, and his wife. His first task was to get the scientific laboratory of 'Insulinde' under way. But the high-flown expectations were soon dashed, for in 1920–1921 a serious economic crisis also developed in the Netherlands' East Indies, and 'Insulinde' did not escape the consequences. Kluyver now realized that his position would soon come to an end; in June he and many other employees were told that they would be laid off in October, 1921.

At this point Kluyver faced two alternatives: either to re-establish contacts with the Department of Agriculture, Industry, and Commerce in Buitenzorg, or to hope for a professorship at the Bandung Technological University where a Division of Chemical Technology was being created; Kluyver had already been interviewed for this position. At this point something utterly unexpected happened: he received a cablegram from the Department of Chemical Technology of the Tech-

nological University at Delft, asking whether he would consent to being placed, as first choice, on the list of candidates for the chair of general and applied microbiology that would become vacant at the end of the academic year 1920-1921 owing to the retirement of M. W. Beijerinck who had reached the 70 years' age limit.

Kluyver was greatly astonished; he had expected that Prof. Dr. N. L. Söhngen, then at the Agricultural University in Wageningen, would be appointed as Beijerinck's successor, and never for a moment thought of himself in that position. With the image of his father before him, he soon realized the attraction of devoting the remainder of his life to science and teaching; but at the same time he recognized the great responsibility he would take upon himself. After careful deliberation he cabled his acceptance.

Kluyver took leave of Java and, with his family, boarded the S.S. 'Patria' at Tandjong Priok on October 26, 1921, reaching Rotterdam at the end of November. Thus ended the 'Indian adventure', as he used to call it.

Those who have read the foregoing account may perhaps be inclined to look upon this period as the story of two more or less unsuccessful ventures; but Kluyver himself was also keenly aware of another aspect. To be sure, there had been disappointments with the activities during these years, but these were counterbalanced by an enormous gain. The 'adventure' had taken him to Java and Sumatra, to parts of Ceylon and of India. He had become acquainted with peoples and conditions in the Orient. This had vastly increased his range of vision, his insight into persons and circumstances, and left a body of experience on which he could draw during a lifetime in Delft.

In his inaugural address of January 18, 1922, he expressed this in his remarks to the student body in the following striking words: '... fate has taken me to far-away countries, and did not Goethe, in his "Wahlverwandschaften", say: "Die Gesinnungen ändern sich gewiss in einem Lande, wo Elefanten und Tiger zuhause sind"? It is true that I encountered neither elephants nor tigers in nature; but the emancipating influence exerted by living in a foreign environment has not in the least worn off.'

Meanwhile the family had moved into the official residence at the Nieuwelaan, initially referred to as 'Beijerinck's house'. Although the view from the front of the house onto the street was somewhat

dreary, the view from the rear, on the canal and beyond, amply compensated for this. Even more so did the splendid garden extending on both sides of the house; the many exotic trees and plants that Beijerinck had introduced elicited also Kluyver's interest and admiration, especially in later years.

A. v. R.

KLUYVER AS PROFESSOR CHRONICLES OF THE LABORATORY

PREPARATION

1922

WHEN, in December, 1921, Kluyver first set foot in his laboratory as professor, it was still permeated with the spirit of his great predecessor. 'Beijerinck', said Kluyver in a 1927 speech, 'was the image of unbridled devotion, nay, utter submission, to research and science which immediately spoke to the minds of the students.' But, apart from respect, that scientist also aroused in his pupils, at least initially, a feeling of awe. When he approached, all discussion stopped. What would be the first thing he would harp on: some minor detail that displeased him about an investigation that was obviously advancing rather sluggishly; or, more trivial though just as frequently, any faint indication of a generally abhorred sloppiness, or the equally detested smell of lingering cigarette smoke? The attitude was coupled with a somewhat uncouth manner of speech; the tone was often uncomfortably direct. However, his successor would soon realize how long and how strongly that influence could make itself felt, even after the departure of this curious personage.

Kluyver found himself facing an immense task. The fact that he had been chosen to occupy this world-renowned chair was generally regarded as the result of a willingness to make a credit appointment. He was considered as rather a layman in the field of microbiology. In the institute of Van Iterson, Beijerinck's most brilliant pupil, he had concentrated on the study of technical botany, and for his doctor's degree he had written an eminent thesis on quantitative sugar determinations by means of yeast fermentations. Although we may take it for granted that in the meantime Van Iterson had acquainted him with the most significant properties of the bacteria, his training and experience in microbiology had certainly not been broad.

The personality and reputation of the new professor were known in the laboratory, and had frequently been discussed; the expectations were not very high. Kluyver sensed a mood of scientific snobbery in the assistants he had inherited from Beijerinck. They were still under the influence of the spontaneous, fickle, and – in spite of his having been born of a patrician family – rather unpolished Beijerinck; now they were confronted with the restrained, sensitive, utterly courteous successor.

In the beginning these contrasts were keenly felt; they were to become even more pronounced.

Kluyver, perhaps subconsciously, assumed that it would be one of his first tasks to transform the late-nineteenth century spirit of the institute into a modern one. The incident difficulties were increased because, shortly after his inauguration, a considerable number of students applied for space in his laboratory. During the post-war years Beijerinck had drawn very few disciples; but this had not worried him in the least. Thus the assistants at the time had come to consider the laboratory rather as a typical research institute, where, for all practical purposes, they should not be bothered with the education of students. As will appear from what follows, this laboratory of the Delft University, whose fame gradually extended beyond the city walls, the country's boundaries, and eventually the oceans, became more and more a world centre of microbiology. No matter how long the duration of the study period had been, or however cursory the visit, it became a 'must' for any self-respecting microbiologist to have been there.

But first of all the internal organization and working methods had to be remodeled. The copying press made place for the typewriter; one card index file after another was introduced. A house telephone was installed; a ban was declared against ringing, let alone shouting, for the technicians. A solemn quietude came to prevail. All of this commanded but little admiration among the staff, especially because the changes did not stop at arrangements instituted by the predecessor. In this respect it was irritating to see that Beijerinck's holy-of-holies, the library, was desecrated by being in part converted into a classroom. Numerous purely botanical journal files unexpectedly wound up at the second-hand dealer's. The garden, with all its exotic plants assembled by Beijerinck, received – or so it was believed – insufficient care and attention.

Gone – although, as later appeared, only temporarily – were the former heuristic discussions at the microscope in which the surprise element, so typical of Beijerinck's attitude, required a perpetual alertness. One missed the instructive walks through the garden, peripatetic quiz sessions during which the hearers were kept on their toes even more because the open air seemed conducive to greater divagations, and thus to further provocative and unsuspected questions. In the lectures one missed Beijerinck's rhetoric and brilliance. The entire picture was different. The new professor was seldom seen behind his microscope. Whenever he was not occupied with the students, he could always be found in his study, surrounded by piles of books. This pattern of behaviour can be understood if it is realized that Kluver did not want to get singed by working on problems he had not yet recognized or, in any case, had not yet mastered. During the evening hours he studied superhumanly. He prepared himself; it is probable that he considered this difficult phase as an incubation period, full of tension, and requiring enormous efforts.

As every one discovered, the reorganization progressed. The assistants saw their erstwhile unlimited opportunity for research curtailed; there were orders to be sent out; the books from the once so magnificent library had to be sorted and assessed as to actual current value, reorganized according to a new system, spread out over a number of rooms to which the students had free access. The pupils increased in number and became ever more of a burden; they took up a hitherto unknown amount of time and care.

Susceptivity to innovations which might prove to be just as good as, perhaps even better than the old order, had long ago been weakened by humdrum; and this prevented the assistants from recognizing how ingenious a man the new professor was. Nor did they see that he considered the time ripe for doing the spadework that would lead to the development of the fundamentals of the 'unity in biochemistry', that gigantic edifice that slowly rose up amidst the venerable temples erected by Beijerinck.

Beijerinck had established himself in his country home at Gorssel. Although he had left Delft for good, and so definitively that he remained unwilling ever again to set foot in it, even on the occasion when Delft solemnly celebrated his golden doctor's jubilee, his influ-

ence nevertheless remained evident. He ordered his senior assistant, Den Dooren de Jong, to conduct various experiments, so that the latter found himself confronted with an insoluble dilemma. On the one hand he experienced the desire to maintain, also by regular visits to Gorssel, a cherished contact that had grown with the passage of time; on the other hand there were the daily activities in the Delft laboratory, in an atmosphere of sweeping renovation. And a choice had to be made. Disappointed, he left the once so beloved laboratory in the course of 1923, with the firm intention, following a higher example, never again to cross its threshold. At the leave-taking, Kluyver's honesty and warm heart made him express regret that his associate had fared so badly during this first year, and declared that he owed him a debt of honour which he hoped to redeem sometime in the future. Den Dooren de Jong likes to tell of the royal manner in which this so-called obligation was subsequently redeemed; how much he owed Kluyver in connexion with the preparation of his thesis, and later with his investigations on bacteriophages which he conducted in Rotterdam; how the personal relationship developed into a strong tie of friendship; and how, to his great joy, his home could offer Kluyver a temporary but welcome and safe refuge during the second world war.

Kluyver, too, visited his predecessor now and then in Gorssel. After such visits he returned not only mentally worn out, but occasionally even disturbed. That he had to digest various quaint, albeit undoubtedly clever, remarks on the most diverse subjects could be tolerated. But he also became aware of insurmountable barriers, as on the occasion when Beijerinck, in reply to an inquiry about a member of the staff, flatly pontificated: 'X is an honest man, but he lies!' A mind as rational as Kluyver's could not stomach this.

Consequently Kluyver could not be blind to the peculiarities of him whom he was wont to call 'my great predecessor'; nevertheless he held him in the highest esteem, and never neglected to make this clear to his pupils. The lecture in which he introduced Beijerinck's elective culture methods under the classical and characteristic title: 'The marvels of a gram of garden soil', usually contained a passage in which Kluyver underscored the significance of the elective culture method as follows:

'Curators of culture collections often receive requests for cultures of

bacteria that can undoubtedly be found on the shoes of the petitioners. It is clear that such microbiologists are more in need of a thorough training in their discipline than of a pure culture of the bacterium they ask for.'

In Kluyver's scientific approach the 'red thread' of Beijerinck's work remained clearly discernable. In later years an unexpected observation often elicited the remark: 'Let's see what Beijerinck has to say about it', and it usually turned out that the latter had already recorded the phenomenon. Kluyver's deep appreciation of Beijerinck's work will be apparent to anyone who peruses his contribution to the extensive Beijerinck biography [1940] under the title, 'Beijerinck, the Microbiologist'.

A final indication of the considerable change that was accomplished during the single year in the guidance of the work and in the atmosphere of the laboratory has its spatial origin outside the institute, though it resulted in the development of a strong inner solidarity. It was the influence of the professor's domicile, an influence that initially manifested itself but timidly.

This house, that Beijerinck had occupied with his sisters, was built under the same roof as the laboratory, and could be reached via the study. It now grew into the beloved home, where the Kluyver family more and more shared the cares of the laboratory, and whose hospitality for associates and visitors was equalled only by the readiness to listen and the open-mindedness that every one encountered in his contacts with the professor.

INSPIRATION

1923-1927

The biographer of these early years has a comparatively easy task in so far as his thoughts scarcely need to wander outside the walls of the institute at the Nieuwelaan. For the events that became the foundation for the later years all took place inside it.

The laboratory and adjoining living quarters dated back to 1895, when the chair for Beijerinck was created. Built in the neo-gothic style of the period, with tall, narrow windows, it had been expanded, in 1911, by the addition of a wing that was, to be sure, better lighted,

but whose soberness harmonized poorly with the other part. It had been intended as a temporary structure; but, like so many temporary things, it was predestined to last for a long time; it was not to be abandoned till after Kluyver's death.

Already the laboratory had been changed from the domain of an imposing scientific Olympian into the more adequate workshop of a teacher whose repute as a man of charming courtesy gradually spread among the Delft students. Nonetheless, it is a striking indication of Kluyver's inspiring personality that it was not his individual attractiveness but the promising tenor of his inaugural address that brought the first of his students – Van Niel – to the door of his study the day after. This example was soon imitated. Thus, in the course of 1923, the laboratory gradually became occupied; at the end of the year somebody was at work in every usable space, from the ground floor to the attic.

The inhabitants enjoyed the atmosphere of a laboratory where there was no longer any pressure of a requisite number of experiments, in anticipation of a final examination; where there was no crowding for space, as in other Delft laboratories; but where they entered into a small community that gradually became closer, and where each found work to do commensurate with his ambition and ability.

Just the same, no matter how much this atmosphere of peace and devotion was respected by all, the spark of inspiration was still lacking. Subconsciously this was sensed in the laboratory where the reverberation of Beijerinck's discoveries could still be heard. But with his departure an era had definitively come to an end; new, epoch-making finds of the kind that Beijerinck had made, could scarcely be expected any more. A few years later Kluyver himself was to formulate this situation by saying that the discovery of a truly novel type of bacteria would cause no less a sensation than that which the 'Loch-Ness monster' threatened to do at that time. Yet it was obvious that a science as young as microbiology stood only at the threshold of its development. Would it be vouchsafed Delft to contribute as much to its expansion as it had done to the foundations? Unquestionably this expectation was alive in the laboratory, but for the time being the activities had to be restricted to making a general survey of what had already been accomplished.

It may have been the unexpected crystals in a glucose-calcium car-

bonate-agar plate, that De Leeuw had streaked with a suspension from an enrichment culture of vinegar bacteria, that initiated the further developments. But let us not ascribe too great a role to chance, of which Pasteur had said: 'In the realm of observation chance favours only the prepared mind'. And here was a scientifically prepared mind that already knew the questions on which were based the gradually ripening answers; a mind, therefore, that was capable of recognizing even in *minutiis* those features that could promote the ripening. In this sense *Acetobacter suboxydans* contributed its share to a process that, once started, rapidly advanced and kept the whole laboratory under tension.

In daytime little was spoken about these developments outside the assistants' lab; at most a casual remark was dropped during the early morning hours, generally spiced by Kluyster's predilection for exaggeration, such as: 'As of last night, microbiology has once more undergone a change of face'. The day was devoted to the students and current matters. In the evening and during a large part of the night the professor and his associate, H. J. L. Donker, ensconced themselves in the study, surrounded by books, and endeavoured to distill true unity out of the diversity of facts and apparently contradictory data. The students, with the exception of a few initiates, at first understood little more than that it must surely be an inspiring labour that called for so much exertion and yet left so few traces of fatigue behind.

As the concepts took on an increasingly definite form, their significance slowly began to penetrate to the students. The expectations grew more and more intense; until one morning the newly acquired insight found expression in the terse, and in its overstatement equally characteristic, phrase: 'From elephant to butyric acid bacterium - it is all the same!' The Unity in Biochemistry had been discovered.

Can anything evoke a greater enthusiasm among the workers in a laboratory than a discovery of this sort, whose scientific importance is so evident? Everyone knew the enthusiasm that Pasteur had managed to awaken in his pupils; everyone was admiringly aware of Beijerinck's pioneering work, particularly because of Kluyster's example. Now all doubt as to whether the work of the 'great predecessor' could be continued had been dissipated; once again a new field had been opened to investigation. What did it matter if at first the extent was overrated? The vast horizon supplied the one element that had thus far been lacking: 'Inspiration'.

What delight it was to work now! A string of candidates for the doctor's degree provided one brick after another towards the completion of the construction of the 'unity'. All of this called for much and painstaking analytical work; the number of fermentation balances assembled during those years is legion. The laboratory was always open, even to those who, because of their position, were not entitled to a key. For there was, after all, the pathway through the door of the director's home, up the stairs, and through the study. Here Kluyver would be found at work, either alone or with one of his pupils. When, the work done, he descended to the laboratory in order to silence his disquietude about the primitive, gas-heated incubators, as he was wont to do every night, he would invariably find a number of his pupils still at work, distilling, analysing, hunting for fermentation products. How keenly he would then catch up with the latest developments! In the middle of the night he would await, with bated breath, the results of an experiment in that building that had already become known as the 'light palace of the Nieuwelaan'. And if the results signified the happy conclusion of a particular investigation, the session was sometimes concluded with a spontaneous rendering of the national anthem.

Although the attention was focussed largely on the biochemical activities of the micro-organisms, a profound interest was nevertheless maintained in morphological and developmental problems. It was during this period that the yeast, *Sporobolomyces*, was discovered; and the antics of *Bacillus funicularius*, curling itself up in strands, were watched with delight by the observers who, stretched out on a sofa, could thus make their observations through an inverted microscope.

This was also the period during which a great intimacy developed between the professor and his closest associates. This always remained controlled because of the respect that his pupils felt for him, and which made it easy for them to accommodate to his courteous style. A contributory factor to this intimacy was the close vicinity of the home; the child's voice that, around 6 p.m., could be heard through the corridor, summoning father to dinner; the encounters with Mrs. Kluyver who so often spent the evening in the study, cleaning up, ordering, writing, seeking an urgent consultation.

And the professor also won the hearts of those pupils who did not belong to this more intimate circle. How amiably he could point out a mistake made by a beginner, correcting without discouraging. The

more advanced group was frequently astonished by the clarity of a verdict that could lay bare the fundamentals of a problem in a terse formulation. Striking, too, was his modesty. When he proclaimed: 'The previous century – the century of Lavoisier – was the century of oxygen; this is the century of hydrogen', it was not said in self-aggrandizement, but out of respect for the work of scientists such as Warburg and Wieland. During a discussion with Söhngen about the merits of Beijerinck and Winogradsky, Kluyver once remarked: 'It is such a pity that only our small group of microbiologists can understand what great scientists they were; what do others really know about them?' Indubitably something of the realization that the founders of microbiology did not always get their due can be gleaned from one of Kluyver's last publications: 'The Microbe's Contribution to Biology', written in collaboration with Van Niel.

The opportunity openly to attest to his admiration for these founders was always welcomed by Kluyver. Already during the first year of his professorship he was in a position to sketch Pasteur's merits in a public address, on the occasion of the commemoration of the centenary of Pasteur's birthday. There we find the trenchant statement that shows how profoundly Kluyver recognized the connexion between the minutest details and a general concept: 'If nowadays bacteriologists all over the world depend on the seemingly so flimsy cotton plugs; if, in their laboratories we can observe the intermittent glow of the inoculating needles; must we not then conclude that these attest to an expression of faith in the fundamental investigations of Pasteur?'

The significance of Beijerinck's work was the subject of a lecture delivered by Kluyver on June 14, 1927, the day on which, fifty years earlier, Beijerinck had received the degree of Doctor of Science at Leiden University. On this occasion, too, a bronze plaque bearing the effigy of its first director was unveiled in the Laboratory for Microbiology. Afterwards Kluyver travelled with a deputation to Gorssel to congratulate Beijerinck personally.

In the place where the plaque was installed had formerly hung a 'No Smoking' sign; this now disappeared. Respect for the 'great predecessor' had caused Kluyver to retain it for more than five years in its original position. But it had been hard on Kluyver, who was an inveterate smoker. And whatever support the professor might count on from his associates, here they completely failed him. A prohibition

that was no longer heeded after midnight had already lost its force. It lost more and more terrain; after 10 p.m.; after 6 p.m., when the younger students had gone; till finally the sign was removed and no obstacles remained in the way of enjoyment of 'poison in one of its worst forms', as Kluyver used to call it.

During these years there were but few foreigners who came to the laboratory, and these only for cursory visits. Undoubtedly it was the old reputation of the institute that attracted them. A special impression was created by the visit of Prof. and Mrs. Lindner on their return to Germany from a sojourn in Mexico where Lindner had studied the manufacture of pulque. The encounter with the great fermentation expert pleased Kluyver, the microbiologist, while in his function as host he was perhaps as much flattered by the real interest of his guests for the spirituous products of domestic origin that he offered them.

No longer within the confines of the laboratory, though still within the borders of the city, Kluyver's first contact with industry materialized; during these years began his collaboration with The Netherlands' Yeast and Alcohol Manufacturing Co., Ltd. This contact had a deeper origin than geographical proximity; it signified the re-establishment of historic connexions between applied and theoretical microbiology.

Van Marken, the founder of the yeast factory, was in many ways a progressive. As early as 1885 he recognized the desirability of scientific investigations of those microbiological processes that lie at the root of the manufacture of yeast; and to this end he succeeded in attaching Beijerinck to his industry for the ten year period, 1885-1895.

Unquestionably Beijerinck's scientific acumen will have benefited the industry through important though indirect contributions. But he definitely was not the sort of person who would unreservedly devote himself to specifically industrial problems, and after his appointment as professor at the Polytechnical College, which later became the Technological University, his contacts with the industry practically ceased.

Viewed from a technical-industrial angle, it is therefore all the more gratifying that, exactly at a time when technical developments were sufficiently far advanced to initiate the stormy growth of a microbiological industry, Kluyver appeared as the man who was so eminently suited to resume the connexion, especially because the studies on the quan-

titative aspects of the various fermentation processes had inevitably led him to consider the possibility of their industrial application.

Consequently a more or less casual encounter with F. G. Waller, Chem. E., at that time a graduate student and later successor to his father as director of the yeast factory, was all that was needed to open negotiations. They led to many years of fruitful cooperation, which became formally established with Kluyver's appointment as adviser in 1928.

The first new fermentation products, manufactured on an industrial scale in 1928, were butanediol and acetylmethylcarbinol. Soon afterwards the latter was used for the production of diacetyl by chemical means. Some years later the manufacture of butanol and acetone was taken in hand. The development, and afterwards the supervision, of adequate procedures called for much technological-scientific work. As a consequence several of Kluyver's pupils found excellent positions at the yeast factory. Kluyver himself paid regular weekly visits to the plant where he conferred with them on the problems they encountered. Especially the butanol fermentation provided material for many discussions and extensive laboratory experimentation. Kluyver's mastery of the literature, both in this particular field and in the most general sense of the word, was exceptionally stimulating, and great was the influence he wielded on the expansion of research work in the entire factory.

In later years Kluyver also established connexions with many other industries that had to struggle with microbiological problems. When informed of their difficulties, Kluyver usually let one of his students conduct experiments on what, in the light of his experience, seemed to be the essential elements of the situation. Most common were the instances, such as food spoilage, anaerobic corrosion, obstruction, etc., in which the industry concerned was plagued by unwanted microbial activities. Naturally, the typical microbiological industries themselves also profited from his knowledge from time to time. Thus the usefulness of microbiological research became more and more appreciated by industry in general, so that gradually an increasing number of Kluyver's pupils went to work in breweries, the dairy industry, sugar factories, water and sewage purification plants, food preservation industries, and even at the Royal Dutch Shell laboratory in Amsterdam, where Kluyver later became microbiological adviser.

While these years of inspiration had yielded many important scientific successes, they also produced another fruit that filled Kluyver with great joy. This was his election to the Royal Netherlands' Academy of Sciences in 1926; and for six years he could attend its monthly meetings in the company of his father. In this august body he established contacts with exponents of the diverse branches of the natural sciences for which the wide interests of his synthesizing mind hankered. And he deemed it an honour to communicate the results of investigations conducted in his institute at the Academy meetings. At a riper age he would be called upon to serve the Academy as its highest functionary.

CONSOLIDATION

1928-1938

The years that followed were happy ones. The work in his institute progressed steadily; more and more frequently Kluyver's attention was solicited elsewhere; and the scientific world took increasing notice of his accomplishments, proofs of which became abundant. But, however much his field of activity expanded, the Delft laboratory remained the nucleus.

Kluyver keenly realized that the passion for work engendered in his pupils would inevitably lead to increased demands for a well-equipped institute. He had succeeded in bringing about some important improvements. The gas-lit incubators had gradually been replaced by new, electric thermostats; a compressed-air line was installed throughout the laboratory. The latter greatly facilitated aeration experiments with the well-known Kluyver flasks [Kluyver, Donker, and Visser 't Hooft, 1925], which up to that time had been used in conjunction with water suction pumps. The new facilities considerably simplified the study of microbial oxidation processes. The kitchen, always an important part of a microbiological laboratory, was expanded.

These changes had been accomplished in spite of a government subsidy which, by present standards, must be deemed very scanty indeed. In fact, it was barely sufficient to defray the running expenses; very little was left for the purchase of new instruments. Hence the material aid of the Rockefeller Foundation, which made it possible to buy various much needed pieces of equipment was most welcome. Nevertheless most things had to be done with home-made apparatus,

so that on many an occasion great ingenuity was required to achieve good results with the primitive equipment. Although the limited subsidy was sometimes a source of annoyance, it nevertheless affected the general atmosphere but little; for deep down in his heart Kluyver found it rather sporting to reach a satisfactory solution with the available funds. With almost fatherly pride he could show visitors what his collaborators had been able to construct with the simplest components.

The division of labour between the members of the staff, now augmented by a second assistant, had not been changed after the first year. Problems of organization, as well as the care of the culture collection, were the primary responsibility of the 'conservator', or associate, who also assisted Kluyver in supervising the work of the students in the laboratory. The assistants were principally charged with the extensive preparatory work that was needed to provide the abundant demonstration material displayed in the lecture courses, and with the preparations for the laboratory course that Beijerinck had originated and that had been greatly expanded by Kluyver. This course was intended to initiate those future chemical technologists who were interested in microbiology in the fundamentals of that science during a few afternoons and evenings per week over a six week period. Candidates for the doctor's degree and special guest workers, some of them from abroad, were of course guided by Kluyver himself. It should be mentioned, however, that this system was not rigorously adhered to; and mutual exchange of ideas and experiences was always warmly encouraged.

Besides the above-mentioned activities, the conservator and the assistants were expected to undertake a scientific investigation that would be suitable for a doctor's thesis. Moreover, the discussions in connexion with the subjects treated in the lecture courses frequently occasioned the projection of special experiments that had to be conducted in addition to the other work.

It was not always easy for the staff members to divide their attention properly over the various activities. Kluyver, too, found the combination of research work, instruction, and organizational chores anything but ideal for his staff. As he sometimes remarked, he hardly dared ask one of his collaborators to have 'his name included among the janitors in the university roster'.

In Kluyver's opinion the administrative and technical personnel

also constituted an important part of the laboratory team, indispensable for the proper conduct of the experimental work. And they, in turn, were just as appreciative of Kluyver's humane and often fatherly guidance as was the scientific staff.

Thus, despite the inevitable turn-over inherent in a university institution, a community had grown up that was deeply attached to the Delft laboratory. But no one manifested a greater attachment than he who was the center of this community.

The rapidity with which Kluyver had made himself familiar with the problems of microbiology, and the mastery he had exhibited, had not failed to make a deep impression in scientific circles. Small wonder, then, that other universities cast covetous glances in the direction of the Delft scientist whenever there was a vacancy in some allied field. As early as 1926 Kluyver received an offer to occupy a chair for medical biochemistry that was being created at Leiden University.

For a long time he weighed the alternatives: Holland's most ancient university had formerly fascinated him, and a close contact with many medical colleagues would undoubtedly have been advantageous to his work; but he also realized that he would miss in Leiden all that he had built up in Delft. Thus he decided in favour of Delft. At about the same time he also declined a call of a different sort, *viz.*, the directorship of the Department of Agriculture in the Netherlands' East Indies.

Some compensation for his loyalty to the Technological University was that students from other Dutch universities now came to join the Delft students in his laboratory. This had been made possible by a new provision whereby certain departments in these universities had agreed to accept microbiology as taught in Delft in fulfilment of the requirements for a minor subject. Soon thereafter the first two Leiden biologists arrived: two girl students who had decided to complete their studies under Kluyver, and who soon found themselves completely at home in the new milieu. Later they were succeeded by many others. Kluyver was always greatly appreciative of this cooperation, especially from Leiden University, where he also served on examining committees; the most important single reason is probably that he thus found the scientific scope and atmosphere of his laboratory materially invigorated.

During the 'thirties' Kluyver was once more faced with a similar dilemma as that which had confronted him a decade earlier. After the

death of Söhnngen, in 1934, he was sounded out about occupying the chair for microbiology in Wageningen. The biologist in him must have carefully balanced the attraction of being able to live in the most beautiful part of his native country against what the vicinity of Delft had to offer; Beijerinck had characterized the environs of Delft as a 'botanical desert'. But once more loyalty to the Delft chair triumphed, probably augmented by the recognition of the ties with the past that Van Leeuwenhoek and Beijerinck had woven between Delft and microbiology. This supposition is therefore the more tempting because this past must have appeared to him more clearly than ever before, as a result of the active part he had taken in the publication of Beijerinck's Collected Works, completed in 1940 with the appearance of the final, biographical volume; and in the planning of a complete critical edition by the Royal Netherlands' Academy of Sciences of all the letters of Van Leeuwenhoek, begun in 1932 on the occasion of the tricentennial of Leeuwenhoek's birth. Kluyver witnessed the publication of the first four volumes of this work.

A final opportunity to change his scientific domicile came after the second world war, from Rutgers University, New Brunswick, U.S.A. Kluyver toyed with the idea of joining in the general trend to migrate to America; but nobody in his immediate environment really believed that it would ever develop into more than an idle contemplation. He had become too thoroughly entrenched in the affairs of his laboratory, of Delft, and of its Technological University. As could have been anticipated, the final decision was again a rejection.

The work in the laboratory, conducted under the banner of the 'unity', progressed steadily. Sometimes, as in the case of redox potential measurements which, it was hoped, would clarify biochemical processes, the important results that were initially expected did not materialize. At other times the work assumed a significance that could not possibly have been foreseen at the time. A striking example is the development, by Kluyver and Perquin, of a method for the submerged cultivation of moulds. This was intended to provide physiologically uniform cell material for the study of the oxidative metabolism of these organisms. Kluyver was very pleased with this elegant procedure, which opened up the possibility of carefully controlled experimentation in an entirely new field. But he was far from suspecting that less than twenty years

later this very principle would be applied on an enormous scale in the new factories that sprang up like mushrooms all over the world.

The stream of dissertations and publications drew more and more attention, now also abroad. The result was that not only graduate students from Dutch universities chose to complete their studies under Kluyver, but foreign guest workers also came to his laboratory.

Soriano arrived from Argentina, in order to learn the methodology of fermentation research; Frateur, from Belgium, made an extensive study of the acetic acid bacteria; some other chemists from the same country sojourned for a time in Delft. Barker there succeeded in isolating methane-producing bacteria in pure culture, and discovered *Clostridium kluyveri*. He was the first of a string of guests from the U.S.A., and was followed by Starkey, Johnson, and Clifton. From Israel came Volcani; from Scandinavia, Hartelius and Wikén, who became Kluyver's successor.

The interest that Kluyver's work had created abroad was apparent not only from the fact that so many foreigners came to work in his institute, but also from the invitations he received to visit universities in other lands in order personally to present his ideas. The invitation to deliver a series of lectures during the spring semester of 1932 at Iowa State College in Ames was, for the laboratory community, the most momentous. He remained in the U.S. from April till the end of August, and, despite a crowded program, he still found time to keep his associates in Delft informed of his experiences.

Kluyver could report that the Delft investigations were greatly appreciated, especially in Ames. The following excerpt from one of his letters illustrates to what extent the publications from his institute had come to influence the workers at Iowa State College: 'The students here can be divided into those who can read Dutch, and the smaller category who cannot. Yesterday I met somebody who was pondering Scheffer's mysterious fermentation balances, and who told me that he would rather read Dutch than German'.

He, in turn, had great respect for American accomplishments, such as those of Buswell in Urbana: 'With this technique he has demonstrated that the queerest compounds can be *quantitatively* converted into carbon dioxide and methane. Phenylacetic- and hydrocinnamic acids are instantly devoured, benzene nucleus and all! Let Mr. de Graaf imitate that as soon as possible; we are shamefully behind!'

And about Madison: 'My lecture on metabolism and redox potential did not seem to be much appreciated; I think that they found this rather "old hat". Besides, my imperfect English improvisation in an enormous hall – 200 in the audience, 1,000 seats! – will probably have contributed to the lukewarm reception.'

From another letter: 'The danger here is that they appear to know the Delft theses even better than I; I had forgotten quite a bit from the older ones. Here they work a lot with Dutch-English dictionaries, and some masterpieces are available in English translation – a precious possession that is not accessible to everybody . . . For the rest, "A prophet is not without honour, save in his own country", and for my American fame it would have been much better if I had remained behind the scenes. Here and there I could notice disappointment with my unassuming appearance, already on first acquaintance', as he wrote to his friend, Van Rossem.

At the time of Kluyver's first American visit the depression was still very much in evidence there. Discussing the economy drive, then also raging in Delft, Kluyver wrote, for example: 'In the U.S. the situation is perhaps even worse because there are so many privately endowed universities. In San Francisco they have recently buried "John Depression", oh simple minds! More important is the fact that the price of hogs has risen, and that this has caused an upswing on the stock exchange. May this reversal in trend be permanent!'

As appears from his letters, Kluyver was greatly impressed by the vigour of the Americans, particularly in the area of scientific investigation. Presumably the respect was mutual; for before his return to Delft Iowa State College conferred upon him the honorary D. Sc. degree.

During his sojourn in the U.S. heavy demands were made upon him; his program was always loaded. Here follows a summary of a 'few days' activities, literally quoted from his letter of June 4, 1932: 'Lecture from 11 to 12; immediately by car to Rockford, a trip that lasted till 10 p.m.; early Tuesday on to Milwaukee, visited the activated sludge plant; that evening a lecture before the local division of the American Chemical Society; early Wednesday on to Madison; visited many laboratories during the day, and had lunch and dinner with the "greats" (Overton and Steenbock); evening lecture before the Wisconsin Chapter of the Amer. Chem. Soc.; Thursday, early breakfast, back by car to Ames, return 6 p.m., seminar at 7.30; Fri-

day, lecture from 11 till 12, off by car to Minneapolis at noon, arrival at 7 p.m.; that evening reception by the members of the Bacteriology Department; Saturday a.m., visits to laboratories (Gortner's, among others), at noon off by car to Hackensack at Birch Lake, in Northern Minnesota – land of the 1,000 lakes, each one of the dimensions of our Loosdrecht lakes – to the cabin of Dean Buchanan; Sunday and Monday spent with the Dean on his lake, fishing (!), canoeing (!), alternating with cooking and dish-washing; Monday evening, 7 p.m., by car to Minneapolis, stopped for an hour and a half with Kolthoff, continued on to Ames, arrival Tuesday at 6 p.m.; at 7.30 my seminar; next morning lecture at 11. All in all, a rather formidable itinerary. Travelled 1600 miles, or 2500 km, in an automobile in a single week!

In his own country Kluyver's counsel in scientific matters was sought with increasing frequency. First love made him accept with alacrity an advisory function with the Governmental Fibre Institute. The tie lasted for many years, and was not severed when this institute became part of the 'Central Organization for Applied Natural Scientific Research' (T.N.O.) in the Netherlands. This is not surprising, for Kluyver had long ago recognized the importance of the sort of investigations that were carried out there. And soon after T.N.O. had been established, Kluyver became a member of the board of trustees of the Central Organization; from 1932 on he served uninterruptedly in this function, and was a member of many of its committees.

On January 1, 1935, began a collaboration that was to require much of Kluyver's attention. The Rockefeller Foundation had made a grant to Dr. L. S. Ornstein, Professor of Physics at the University of Utrecht, for the establishment of a group that would be concerned with investigations of problems in the area of biophysics. Ornstein associated himself with Kluyver, and under their combined guidance the Biophysical Group Utrecht-Delft came into being. From the memories of E. C. Wassink*, for many years an associate of this group, the following picture of Kluyver's activity in it emerges:

* The following account is a slightly adapted version of an article of Dr. Wassink, kindly submitted by him to the Editorial Committee. The work of the Biophysical Group Utrecht-Delft has been reviewed by Wassink in: *Advances in Enzymol.* 11, 119, 1951; and by Spruit and Spruit-van der Burg in: *The luminescence of biological systems*, edited by F. H. Johnson. Washington, 1955, p. 99.

'At the start the group concentrated on a study of bacterial luminescence and photosynthesis, in a manner that would guarantee the closest possible collaboration of physicists and biologists. It was housed in the Physical Laboratory of Utrecht University, which, in 1938, was expanded by the addition of six new rooms for the group, made possible by a generous new subsidy from the Rockefeller Foundation. Although Ornstein supervised the daily activities, the younger members did not hesitate to communicate with Kluyver whenever they had obtained significant results, either by writing to him, or by visiting him in Delft, especially on Saturday afternoons. The most important contact between the group and its Delft leader was, however, maintained through Kluyver's periodic visits to Utrecht, which occupied an entire day. These visits were always stimulating, strenuous, courteous, and friendly. The association of the two leaders, each a world-renowned authority in his own field, was particularly suitable to inculcate into the younger workers an appreciation of what is required of scientific work that is worthy of being judged by international standards.

'On such days the experimental work was usually suspended, and the group assembled in Ornstein's room for extensive discussions, while enjoying coffee and tobacco. The discussions were interrupted only for luncheon, in a nearby restaurant that was favoured by Ornstein. Kluyver used jokingly to regret the interruptions, and on one such occasion he discoursed, in science-fiction fashion, on the beneficent effects of food tablets that would immediately restore one's capacity for work for several hours, and could be carried in goodly quantity in a waistcoat pocket. We may assume that Kluyver, despite everything, found it quite appropriate that the deprivations of war made it necessary to replace these luncheons by a simple meal, consisting of a few slices of bread and a tea- or coffee substitute, eaten in the laboratory.

'At the start the two leaders had agreed that their names should not ordinarily appear on scientific publications by the group. Only once did they deviate from this principle. Because the publications were primarily biological, Kluyver took an active part in their preparation. He spent many weekends with the authors, whose experience in this respect was identical with that of Kluyver's pupils.

'Ornstein died in 1941; his end was undoubtedly accelerated by the cruel measures of the occupation. Under the difficult circumstances of wartime, Kluyver directed the program single-handedly until

Milatz was appointed as Ornstein's successor. Milatz resembled Ornstein in many respects, and contributed a generous dose of optimism and enthusiasm which was much appreciated and helped to cheer up Kluyver during his visits. Thus the work was continued, even though the Rockefeller grant had ceased, until the critical situation during the winter of 1944 caused it to stop almost entirely.

'After the end of the war the experiments were soon resumed. But the frequency of Kluyver's visits to Utrecht gradually diminished, especially during his last years. Milatz too found less and less time for the group because he was involved in the establishment of nuclear-physical studies.

'In general, the close collaboration of a physicist and a biologist, envisaged as an ideal during the early period, receded into the background.

'About the time of Kluyver's death, Milatz left the physical laboratory, so that the group was robbed of both its leaders. Till now they have not been replaced, and probably will not be; the present senior workers continue the work independently. One reason for this situation is surely that it would be difficult to find another team of leaders that could arouse the sort of scientific fervour that Kluyver and Ornstein engendered.'

Kluyver's capacity for work and intellectual ability were devoted not only to science and technology. It was particularly characteristic of him that requests to serve the community in functions of minor importance were never refused. Thus, for many years, he served the city of his domicile as a member of its sanitary commission, whose task it was to advise the city council in matters of hygiene. Such work did not always give him satisfaction; he was, for example, considerably disturbed when, against the commission's advice which he fully supported, a cemetery was expanded in an area where, thirty years later, the new buildings of the Technological University were erected. Nevertheless, such experiences did not prevent him from taking upon himself other duties, such as the function, first of trustee, later of president of the board of trustees, of one of the local secondary schools.

As his age advanced, his associates, and no doubt his children too, often advised him to give up such time-consuming activities; but he could not be moved to do so. He was probably convinced that in a

democratic community everybody should take part in matters of the common weal to the full extent of his ability. This, by the way, was also Mrs. Kluyver's stand; she too had always found time for social work in addition to caring for a growing and developing family.

When in 1955 Kluyver, at his own request, was retired as president of the board of trustees of the secondary school, the Mayor of Delft presented him with the honorary silver emblem of the city; this was also intended as a posthumous appreciation of the considerable social contributions of his wife who had recently died. With this homage the city council gave a striking tribute to the civic-mindedness of both.

Thus, at the end of the 'thirties', Kluyver led a life of harmony, rich by virtue of a close contact with much that fascinated him in science and society, both here and elsewhere. His fame had been established, and his career had reached a summit.

On May 25, 1939, the occasion of Kluyver's quarter-century doctorate, 'Chemisch Weekblad' published a special issue. His teacher, Van Iterson; his colleague, Van Niel; and his pupil, Kingma Boltjes, each from his own vantage point, contributed essays that served to create an impressive balance of all that Kluyver had given during these twenty-five years to science and to his pupils. Yet, rather than presaging a continued assent, this commemoration turned out to be a turning point. The war rumble could already be heard distinctly; the 'good old days' were at an end.

WAR AND OCCUPATION

1939-1945

Pupils from the 'twenties and early 'thirties remember that Kluyver's vocabulary contained such favorite slogans as 'Never say die', 'Keep smiling', 'Some day we'll know'; but during the last years before the outbreak of the war another one came to the fore, the distinctly pessimistic 'This is a great and terrible world'.

The unmistakable threat of what was to come depressed Kluyver perhaps more than others; but as long as it was still possible, the work was continued in the race against time. Kluyver was about to embark for a trip to New York, where he was to participate in the third International Congress for Microbiology, when the threat of war interfered;

the news reports were so ominous that he felt obliged to cancel his journey. On August 27, 1939, he wrote to Van Niel:

'What hurts most is that now the plans for our reunion have come to naught. Believe me that the three months of vainly spent efforts in preparing for the congress and the tour that was to follow – on Thursday 240 lantern slides had been carefully packed in my suitcase – do not grieve me as much as the lost opportunity to see and talk with you again. The congress itself and the many lectures in numerous places that had been scheduled for the subsequent tour of the U.S. have never appealed to me; in any case, the superabundance of contacts would have rather got me down. *Qui trop embrasse, . . .*

'Above all else I realize, however, that personal regret about the course of events sinks into nothingness in view of the tortures to which millions of people are subjected at this moment.'

On May 10, 1940, the Germans invaded Holland; as soon as the immediate turmoil of war had subsided, Kluyver called a meeting of the laboratory staff in order to consider the new situation. He expressed his deep-seated conviction that in the long run right would eventually prevail, and he urged everyone to resume his work. The meeting was concluded with the national anthem.

Now began a period during which the laboratory population steadily declined; numerous necessities began to run out; and the contacts with foreign countries were broken off more and more.

On the other hand, there also occurred a distinct change in Kluyver's mental attitude towards his work. He was, after all, not the kind of scientist to whom his occupation means everything and who could work in total isolation, divorced from any and all ties with social events. His speculative mind, instead of concentrating on the work, strayed increasingly towards the experience of foreign occupation and war violence, alarming and laming by their brutal and chaotic irrationality. The news bulletins broadcast by the allies were faithfully taken in. The creative scientific work no longer progressed in a mood of playfulness, and often had to yield to activities concerned with the practical requirements of the moment.

During the war days the laboratory and the home were a natural refuge for personnel, staff, and neighbours who were received with great hospitality and could seek protection in an improvised hiding trench. After the bombing of Rotterdam the laboratory was charged

with the bacteriological control of the drinking water which was supplied by that city, while Mrs. Kluyster had an important share in locating shelter for the refugees who had come from there.

The laboratory garden was used for the cultivation of potatoes, peas, beans, and, not to be forgotten, tobacco. The scarcity of the latter product hit Kluyster particularly hard, and it must have been a great satisfaction to him that he could at least apply his knowledge of the tobacco fermentation in a directly useful manner.

Agar could, of course, no longer be obtained from abroad. This served as a natural impetus to examine more closely the potentialities of silica gel as a substitute. Furthermore the bacteriological manufacture of lactate, used as an additive to fodder, was taken in hand in view of a threatening depletion.

But in addition Kluyster still managed to devote his attention to dissertations and publications that were in preparation. During this time the sixth volume of Beijerinck's 'Collected Works' was completed, as also the section for the Dutch 'Textbook of General Botany' which he wrote in collaboration with Wassink, and a few papers that were published in 'Antonie van Leeuwenhoek', in English. He also contributed much to the writing of the second volume of the classification of the non-ascosporogenous yeasts.

The cares weighed too heavily, however; no longer could a preoccupation with such activities bring solace. It was only through his iron sense of duty that he could bring himself to perform them, and even so the tempo was greatly reduced during these years. The device of William the Silent, 'Point n'est besoin d'espérer pour entreprendre, ni de réussir pour persévérer', which he had many a time held up before his pupils, now had to serve as an inspiration to himself as well.

Kluyster's loyalty towards his country was equalled by that which he experienced towards what were then the Dutch East Indies. During his early years as a scientist he had been so profoundly impressed by this country that the Japanese occupation of this realm shocked him greatly. And his attachment to the Technological University, where he had been a student and later a professor for so many years, caused him to suffer as a personal attack the measures of the occupation forces against the Dutch universities. The dismissal of Jewish professors by the Nazis, later followed by the deportation of students who had refused to declare their loyalty to the occupant, almost completely lamed

any instruction. He was often consulted about the difficult decisions that the Technological University had to make during these years, and his voice carried great authority.

The role of leader in the resistance against the occupation was incompatible with Kluyver's character; but he was often a trusted adviser to those who fought the German attacks on students and universities. He was deeply moved by the fate of hundreds of students who had been deported to Germany as forced labourers, and he established a bureau that endeavoured to remain in touch with each one of them. By amassing information he succeeded in greatly improving the deficient contacts with parents, and he acquired a fully documented knowledge of the regrettable conditions under which work was being done in many camps and industrial plants. This caused a strong protest to be launched with the German authorities; alas, it was of no avail. In many instances, only too frequently of a tragic nature, Kluyver could lend moral support.

The yeast factory, too, experienced hitherto unknown difficulties. Kluyver learned about them when, in his capacity of adviser, he paid his weekly visits to that community which consisted so largely of his own pupils. But these visits were bright spots in his existence as well; occasions on which he could share with his former coworkers such simple pleasures as the daily cup of soup that was served in the plant in those times of severe food rationing. And it filled him with pride that during the last years of the war, when rumours about penicillin had begun to penetrate from allied sources, a research team at the yeast factory had managed to prepare batches of this 'wonder drug' of such purity that they aroused Sir Alexander Fleming's unstinted admiration.

Anxiety about his own family added to all the other worries of these troubled times, and Kluyver's vitality and health were progressively undermined as a result of the food scarcity. Now and then he was forced to go into hiding or, with great bodily exertion, to provide food for his family. Delft was situated in the area known as 'Fortress Holland', where, during the hunger winter of 1944-1945, the horrors of war ran rampant. Nevertheless, weakened and emaciated as he was, he succeeded, with the aid of his faithful technician, Veenhoff, in maintaining the valuable pure culture collection almost fully intact, and this under circumstances that necessitated the improvisation of

even the barest essentials. By that time the work in the laboratory had obviously come to a complete standstill.

RECOVERY; THE FINAL YEARS

1945-1956

The liberation, dramatically ushered in by the famed allied food droppings, brought a welcome relaxation. With joy and gratitude Kluyver realized that his family and laboratory had come through the struggles unharmed. But he could not be blind to the total collapse of the fatherland, and to the immense problems that cried out for a solution. More particularly, he realized that a vast deficit had developed in Europe in the field of scientific endeavour. 'Beijerinck has made Dutch microbiology great; but now we have been left utterly behind'; this is how he sized up the situation. And this view was strengthened when he could gradually acquaint himself in some detail with what had been accomplished outside the occupied territory.

One of the first contacts he established with the outside world was with Sir Jack Drummond, who had arrived in the Netherlands with a food team. Soon afterwards news began to flow in from America, largely in the form of reprints collected for him by his friends during the war years. He was deeply moved by the fact that in the U.S.A. a 'Delft Library Fund' had been created through which the laboratory could acquire numerous books and journals. Perhaps the most striking token of sympathy came from Mr. Ben May, in Alabama, who provided funds for the purchase of a Beckman spectrophotometer. In his own way, Kluyver honoured the donor by attaching a sign with the inscription 'Ben May Institute' to the door of the little room where the instrument was installed.

With the aid of his coworkers, enormous quantities of reprints were sorted in the large class room. This operation progressed slowly, for Kluyver could not resist the temptation to browse around in, and comment on, this literature, and it was a valuable experience for his pupils. Avidly and admiringly Kluyver let the new knowledge sink in; the lectures were immediately brought up to date; and students and assistants were put to work, repeating various experiments in order to familiarize themselves with the newly developed techniques.

Kluyver reacted to this sudden gush of new information in a man-

ner similar to that which he adopted when he had come to Delft as Beijerinck's successor. Once again he chose not to follow the road of narrowing and restricting his attention to some specialized topics; once again he set himself the task of assimilating the new knowledge in its entirety, frequently consolidating his critical conclusions in papers in which the recently opened fields were reviewed.

Already at a relatively early date, in 1949, Kluyver found an opportunity personally to re-establish some of the broken contacts when he made a trip to the U.S.A. on behalf of the yeast factory. Building on the experience gathered during the war in the manufacture of penicillin, this concern had greatly expanded its activities in the pharmaceutical area, a development which had led to the appointment of a group of medical advisers, and Kluyver naturally became president of this advisory board.

Meanwhile an entirely new methodology for the study of microbial morphology had begun to take shape in Delft. Just before the war, electron-optical developments abroad had led to the appearance on the market of the first electron-microscope. Surmising its great potentialities for biology, Kluyver and Mr. F. G. Waller of the yeast factory joined forces and obtained the necessary material aid to enable Professor Dorgelo from the Department of Technological Physics to push his investigations in this field. During the war one of the students in that laboratory succeeded in constructing clandestinely an electron microscope that was regarded as the best in existence; and soon afterwards this instrument was in use every hour of the day, for the examination of diverse objects, including many biological preparations. Before long it became feasible to set aside a portion of the available time for microbiological studies, and eventually the microbiological laboratory acquired its own electron microscope. Thus Kluyver's strategic outlook had promoted, and in a particularly satisfying manner, a development that resulted in important pioneering work in the field of microbial morphology.

As is evident from the bibliography and the list of dissertations, the scientific investigations in Kluyver's institute were rapidly resumed. The study of inorganic hydrogen acceptors was expanded by further work on the reduction of carbonate and nitrate. Moreover, the results of studies on cellulose decomposition in the rumen of cows attracted

particular attention; these led Kluyver to remark that the rumen of herbivores should be considered as a large fermentation vat.

As formerly, pupils came from Leiden and Utrecht, as well as from abroad. Fåhræus, Nickerson, De Ley, Erkema, Cantino, Van der Walt, Kistner, Pontieri, and Battley conducted experiments, each on a different subject. Besides, a change in the curriculum of the Technological University now required students who wished to specialize in a biological area to spend an additional six weeks in Kluyver's laboratory, during which they were to undertake a study of a special problem. Frequently the associate had to marshall all his resourcefulness in order to provide adequate space for the many applicants. A further handicap was the fact that in the post-war years it was extremely difficult to obtain various laboratory utensils. About 1950 Kluyver decided to have his staff – now increased by the addition of an assistant-in-chief, whose title was later changed to that of scientific officer – conduct the laboratory courses. Furthermore, after the war a full-time assistant was appointed to take charge of the culture collection. Kluyver continued to pay close attention to the maintenance of an adequate instrumentarium; and the laboratory acquired, for example, the equipment needed for tracer studies. But soon there was not enough space left to install additional apparatus.

On January 18, 1947, Kluyver had occupied the chair for microbiology for a period of 25 years. The 'Netherlands' Society for Microbiology' issued on that occasion a special Jubilee Volume (Antonie van Leeuwenhoek, Vol. 12) with numerous contributions by friends and admirers from all over the world. It took much gentle persuasion before Kluyver finally agreed to a small celebration in commemoration of the event; but the Saturday evening on which this took place was for him a truly happy occasion. Nearly all his collaborators of the past 25 years were present. The high light was indubitably the speech which the celebrant himself delivered. It took nearly two hours; he left out nothing and nobody, and recalled numerous details which clearly revealed that the element of personal interest generally dominated the contacts he maintained with his former pupils.

Since the pre-war years Kluyver had undeniably changed. The 'eager young tiger' had gradually developed into one of the 'grey eminences' of science, upon whom many honours were bestowed, and who was more than ever called upon to deliver public addresses and

to undertake assignments of longer duration. The world around him, too, had become a very different one; and in the wake of the upheavals many new problems had arisen that demanded solutions. It was evident that these should be formulated by persons who were acutely aware of the changed conditions and of the events that had caused them. Above all, the war had emphatically underscored for him that science can have only relative significance, and represents but one of the many factors in the composite that governs all human endeavour. He felt that his position and gifts compelled him to accept responsibility for more than the mere pursuit of his special field of science.

Perhaps he realized this obligation all the more strongly because it was one of the attractive features of his chair that he could devote his activities almost entirely to the education of only a small group of truly interested pupils. Thus he was privileged in comparison to many of his colleagues who had to spend most of their time and efforts in the elementary training of large numbers of students.

Consciously he now increased the sphere of his activities in the wide area of his interests that had already begun to unfold before the war. He continued to guide the scientific work in his institute; yet he also bore witness to the outside world of all he had gained in knowledge and insight during his rich life. His initial pessimism, which occasionally expressed itself in extremely sombre moods, and was caused by the recognition that he had fallen behind in his science, further aggravated by the great needs of the country, gradually dispersed; eventually it was superseded by the constructive interest he took in the great problems that were in store.

When the need for remodeling the system of higher education was felt in the Netherlands, Kluyver was appointed to the State Commission that was charged with recommending measures that should be taken to achieve the desired goals.

In 1947 he became Secretary, and a year later Rector Magnificus of the Technological University. The authority and respect he commanded in the academic senate, and particularly among the students, were unrivaled. He knew how to win over the younger generation to his aims, and felt completely at home among the students. He was hospitality personified when officers of student organizations called on him, and during the existing housing shortage he set a personal example by making an apartment available in his home.

Obviously this aspect of his rectorate gave him much satisfaction, for 'What can be more beautiful than to live in the hearts of the younger generation?', as he wrote to a friend shortly before his death.

He had also been elected to membership in the Council of the Royal Institute of Engineers. In this, as in many of his other official functions, he was frequently confronted with various problems that had their origin in marked changes in the social environment. These changes had made higher education accessible to large numbers of young people from non-intellectual milieus. At the same time, the country's economy called for an intensive industrialization for which, in turn, many university graduates were needed. The task devolving upon the Technological University, of educating 5,000 instead of 2,000 students without lowering standards or abandoning tried traditions, was in fact an impossible one. But Kluyver threw himself into the problem with his accustomed thoroughness, and amassed facts and ideas from every side. He frequently referred to these problems in discussions with his associates and students in the laboratory. Although this caused a momentary interruption of the flow of the scientific work proper, it contributed to the participation of his pupils in the real problems of the moment. It was a great support for him that his wife had developed a similarly directed interest in the existing social problems.

From 1947 till 1954 Kluyver was President of the Natural Sciences Section of the Royal Netherlands' Academy of Sciences. His feeling for history led to the restoration of the proud headquarters of the Academy, the 'Trippenhuys', during his presidency. Moreover, there were problems of reorientation conditioned by the times, and Kluyver was too much attached to the Academy to hesitate in placing all his ability and energy at its disposal. With dash and dignity he represented Dutch science, compelling respect for his thorough knowledge. An example of the manner in which he acquitted himself of this task may be found in this book, where the after-dinner speech, 'An Aspect of the Promotion of Science', delivered at the annual meeting of the National Academy of Sciences in Washington in 1953, has been reprinted. It was the second time that a foreign sister institution, on this occasion the Netherlands' Academy, had been honoured by an official invitation to attend such a meeting.

Kluyver had already served the Academy in numerous committees, among others in the Leeuwenhoek Committee. During his presidency

he was appointed, as Academy delegate, to the chairmanship of the 'Commission Kluyver', which was to prepare recommendations to the Government concerning atomic energy investigations in the Netherlands. His forward-looking attitude and proficiency as a mediator were to set the tune in this commission. When the 'Netherlands' Reactor Centre' was established, he was made a member of its executive council. Shortly afterwards, on invitation by the Government, an Academy committee under his leadership studied the dangers to life connected with the detonation of atomic weapons. Although it is certain that Kluyver owed these appointments to his great authority and diplomatic skill, it is worthy of note that in his 1922 inaugural address, reprinted in this volume, he had already anticipated that man would some day be able to exploit the enormous intra-atomic energy.

In post-war years, as mentioned earlier, Kluyver was often invited to deliver special lectures. Once he had accepted such an invitation, he considered it beneath himself to live on past glory, and felt obligated to contribute the best that his experience could offer, always adapting the topic and presentation to his audience. This involved an extremely thorough and time-consuming preparation, which extended over weeks and used to continue till the very last moment, although it was always completed in time. A glance at the bibliography and the list of honours will suffice to indicate the number and variety of this sort of lectures, which often involved fatiguing journeys as well.

These and the previously mentioned activities must be superimposed on the regular daily schedule which, in these days, bore a truly kaleidoscopic character in its own right. Problems of instruction in the Department of Chemical Technology, committee activities in his own field, preparation for congresses and other scientific meetings, weekly and monthly visits to industrial concerns for which he acted as adviser, incidental requests for advice on technological or scientific matters, and naturally, the direction of the work in his laboratory, the lectures, and the examinations, these represent the most important categories into which his work might be classified. In addition there were separate and special occasions that required his attention, such as the voyage to Trondheim in 1954, where he took part in the promotion of the Norwegian microbiologist, H. Larsen, who had conducted his work for the doctor's degree under Van Niel.

Another such example was the selection of Kluyver as promoter of

H.R.H. Prince Bernhard of the Netherlands when the latter received an honorary doctorate in 1951. This homage on the part of the Technological University, to which Kluyster felt so deeply attached, must have been very dear to him, just as was another mark of distinction, which the Government evinced when it donated an authentic Leeuwenhoek microscope to the Technological University on the occasion of its semi-centennial.

It is not surprising that Kluyster often began a conference with a plaintive 'Life is complicated!' Nor is it astonishing that now and then he felt that his pupils did not receive their due, which made him limit the time for his own rest to the barest minimum. In this way he kept a firm grasp on the activities of his students and staff. And, although the personal contacts with the students may have appeared somewhat scanty in comparison with the situation in the thirties, it nevertheless remains true that they were both more numerous and more intensive than those which the students were apt to experience elsewhere in the university. Besides, the previously mentioned tendency to make his pupils partners in the manifold problems that occupied him added further to their unique educational opportunities.

It goes almost without saying that, under the circumstances sketched above, Kluyster's amazing physical endurance could not stand up against the demands of his mental activities. For a number of years prior to his death he had been suffering from angina pectoris, but nothing could induce him to spare himself. He continued to smoke incessantly, albeit that he occasionally shifted to Egyptian cigarettes and limited himself to only one cigar per day. His perseverance was magnificently evident in 1953 when, during a rough crossing on his way to London, he and his chair tumbled down a couple of iron staircases on board ship. He suffered serious injuries, from which he did not recover till many weeks afterwards. Nonetheless, on the day after his arrival, and covered with bandages, he delivered the Leeuwenhoek Lecture before the Royal Society. A member of the audience has described this feat as 'one of the most heroic performances' he had ever witnessed.

That in spite of all this he could keep up this extremely busy life for so long was largely owing to the care with which his wife surrounded him. When she took ill a shadow fell over Kluyster and the laboratory, for she too had occupied an important place there. During

the post-war years it was she who saw to it that the ever-changing ranks in the personnel were regularly kept filled. She also played an active part in attempts to solve the housing difficulties of the staff. In fact, she was consulted whenever human problems arose in the laboratory, and discussions between the professor and his staff about such problems always ended in conferences with the 'unsalaried chief of personnel' of the institute. Thus, when she died in 1952, it was also a severe loss to the laboratory. It hardly needs commentary that the next few years were particularly difficult for Kluyver; during this time his children proved a great support to him. But the most important consequence, the terrifying spectre of the lonely future that awaited him upon his retirement, was something of which nobody could relieve him; and there was irony in the circumstance that, just at that time, the plans for the new laboratory with adjoining living quarters began to assume a definite form. He had long looked forward to this development, and now he would scarcely be in a position to enjoy it any more.

At the outbreak of the war, 'the institute that had been promised me as early as 1924' had not progressed much beyond the stage of a rough draft. When, after the liberation, funds were made available for newly equipping the Technological University, Kluyver hoped that now his new institute would soon come into being, so that he would still be in a position to use it for at least six or eight years before handing it over, completely equipped and functioning, to his successor. With enthusiasm and thoroughness he had helped in the drafting of the plans; he had consulted architect friends and foreign acquaintances; he had gathered documents and designs of other laboratories and during his travels he had collected every pertinent datum of information. But now it was a problem of financing, then again the city's planning commission, that caused postponement or required modification of the plans. What had once been a joy gradually became a lingering burden, and with the passing of the years it finally was only a sense of duty towards the chair for microbiology itself that could still act as a stimulus. In addition, the impending departure from the old and familiar surroundings at the Nieuwelaan seriously troubled Kluyver, so that, when at long last it became evident that even under the most favourable conditions he would profit from the new institute for no more than a couple of years, he sometimes could

not resist the temptation to complain, during meetings with the building committee, that 'the last paper of a scientist is the description of his new laboratory', although this did not diminish the gratitude with which Kluyver greeted the plans. The first pile was driven into the ground in 1955, and in the end the new institute, built according to Kluyver's specifications, was not ready for occupancy, as a precious heritage, until 1958.

In 1954 he returned enthusiastically from his extensive tour of the U.S.A. where, jointly with Van Niel, he had delivered the Prather lectures at Harvard University. The common European impression, that the supposed supremacy of scientific research in America need not be taken seriously, he denied emphatically. He was full of admiration for the American achievements in his own field, and searched for the secrets that could explain the success of those research institutions. Now that the authorities had made available the bricks for the new building, Kluyver occupied himself with the question how, in the light of his recent experiences, it could be appropriately staffed with brains. The problem of equipping the new institute with modern apparatus too had occupied him during his travels, and the purchasing plan he formulated in 1955 greatly benefited by the information he had gathered.

Van Niel's visit to Europe, during which he spent several lengthy sojourns in the Delft laboratory, acted as a strong and refreshing stimulus. There was ample time and opportunity for extensive discussions, not only between the master and his former pupil, but also between Van Niel and the coworkers in the laboratory. The return of the associate, Verhoeven, who had spent a year in the U.S.A. as a Fellow of the Rockefeller Foundation, contributed further to the anticipation of modernization and of the fresh start that Kluyver would initiate in the new institute.

The tragic element that scarcely came out into the open was, obviously, that both Kluyver and Van Niel prepared a new future for Delft microbiology in which neither of them would play a part. It had penetrated to only very few of those who daily surrounded Kluyver that only mental energy kept him going. But pupil and master realized that this was to be their last meeting, and the farewell in the spring of 1956 was difficult for both.

Kluyver did not fool himself as to his state of health, and occasionally intimated that he reckoned with the possibility of not personally participating in the developments of the near future. When, in rapid succession, the fathers of two members of the personnel died from old-age complaints, he expressed the hope that he himself would pass away amidst his work, and in full possession of his powers.

On Saturday, May 12, he attended the annual meeting of the 'Holland Association of Sciences' in Haarlem; the next day he was occupied with matters concerning the future organization of the laboratory, working as usual till late at night. During the first hours of May 14 the end came.

Especially during the last year Kluyver had frequently been occupied with problems of life and death. In his lecture, 'Microbe and Life', which he used to call his 'swan song', he had expounded his ideas on the origin of life. While working on another lecture, which he delivered on May 9, 1956, before the Delft student organization 'Vrije Studie', he indicated to his associates in the course of several discussions that he would again touch upon his views on the problems of life. It was evident that the preparation for this lecture weighed heavily on his mind. But when he began his discourse in the old library of the student union which held so many memories of his own student days, he instantly captured his youthful audience, and it was touching to experience how his devotion succeeded in transmitting, seemingly in the form of a dialogue, the ripened conclusions of his life-long searchings to those whose careers still lay before them.

Two days later he reported, as was his wont, to his scientific staff in the laboratory; at that time he also mentioned that his heart had troubled him considerably during the lecture, and that he had even tried, unsuccessfully, to consult his physician the next day. This was all the more remarkable because he never talked about his state of health, and immediately sidestepped any remark launched by others in this direction. Although he knew that dinner was waiting, he continued the discussion, which presently returned to the subject of the lecture. After having talked about the evolution of the micro-organisms, he turned, as if inevitably, to the influence of environment on man and, in support of a remark, he reiterated the example used in one of his earlier lectures, showing that Darwin and Lincoln, born on the self-

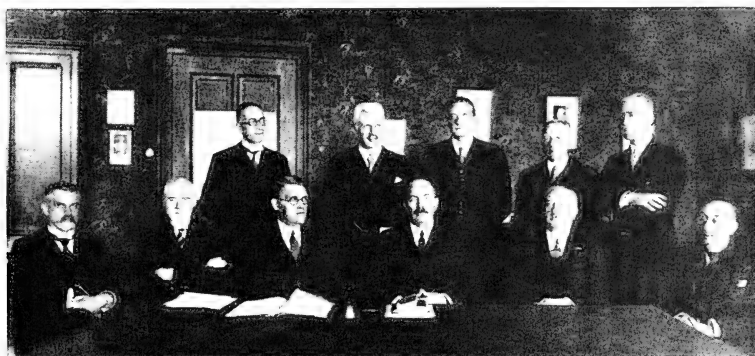
same day, owed their eminence in part to their environments; had they been exchanged right after birth, the world would have suffered the loss of a great biologist and a renowned statesman. It was impossible to escape the impression that he indulged in this scientific discourse in order to express what was occupying his mind, and that he was actually applying his evaluation of the comparative significance of genetic heritage and the vicissitudes of life to himself.

It was already well past 7 p.m. when his associate accompanied him to his house through the long corridor and the room where the yeast collection was stored. 'Tomorrow I won 't be here; I'll see you again on Monday' . . .



1. The young professor: 1926.

2. Kluver amidst his colleagues of the Chemistry Division: 1935.



indicated to give due attention to the pH of the medium, and it soon appeared that a removal of the pigment from the water phase was only advisable, if the medium was ~~not~~ first acidified. The addition of some hydrochloric acid to the ~~medium~~ ^{to the medium} changed the colour range of the culture into yellow, which may be a more extensive precipitate is formed. These crystals dissolved in alcohol, giving a bright yellow solution. The pigment is again removed from the alcohol by washing with distilled alkali; ~~the~~ ^{the} ~~alkali~~ ^{alkali} ~~is~~ ^{is} ~~used~~ ^{used} in this operation the colour range of colour is restored. After reacidification a second extraction with chloroform, with chloroform can be carried through. The chloroformic solution is free from lipids, since these have ~~not~~ ^{not} ~~been~~ ^{been} ~~obtained~~ ^{obtained} in the first chloroform batch. After evaporating the chloroform ^{with} ~~the~~ ^{the} ~~product~~ ^{product} ~~is~~ ^{is} ~~now~~ ^{now} ~~in~~ ⁱⁿ ~~the~~ ^{the} ~~final~~ ^{final} ~~chloroform~~ ^{chloroform} ~~solution~~ ^{solution} ~~was~~ ^{was} ~~dried~~ ^{dried} ~~overnight~~ ^{overnight} ~~with~~ ^{with} ~~anhydrous~~ ^{anhydrous} ~~sodium~~ ^{sodium} ~~sulphate~~ ^{sulphate} and the chloroform concentrated by ~~evaporating~~ ^{evaporating} the chloroform in vacuo. Evaporation of the last chloroform always led to crystalline pigment product.

The product was usually recrystallised in ethanol (95%) till the crystals showed a constant melting point.

It soon became evident that the products obtained according to this simple procedure showed marked differences. This was at once perceptible from the rather pronounced differences in ~~colour~~ ^{colour} of the culture media, which difference was also manifest in the final products. Moreover, this difference obviously correlated with differences in melting point and in crystal shape.

Amongst these there were two products which ^{apparently} prevailed: pigment I which was obtained in fine needles with a melting point varying between 23° and 241° C. (uncorr.) and which dissolved with a yellow colour in dilute alkali, and pigment II which showed orange-red to bright red irregular crystals ~~which~~ ^{which} on heating showed a sintering at 209° C. and melted in the neighbourhood of 225° C. However, it should be added that in other cases preparations showing a different shade and a different melting point were obtained.

It is remarkable that the interesting conclusion is that at least two, and possibly even more pigments can be formed by the organism in question.

This is in a certain contrast to the

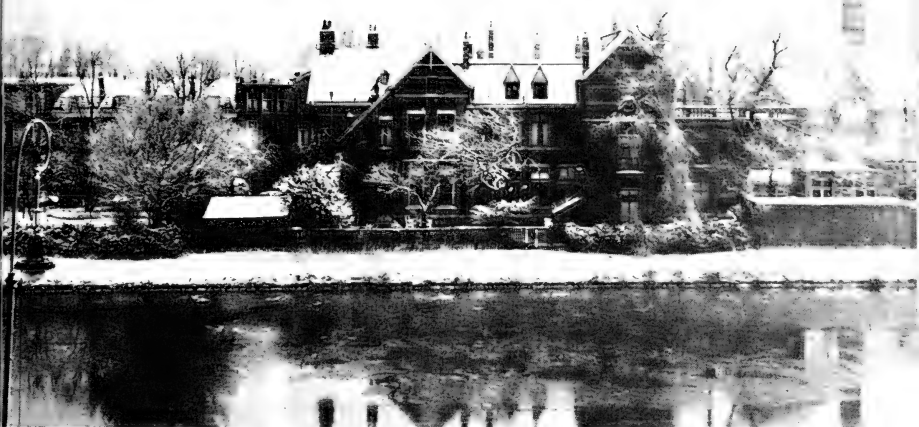
character of the pigment which is distinctly different. In the present case the

Distilling

after several times

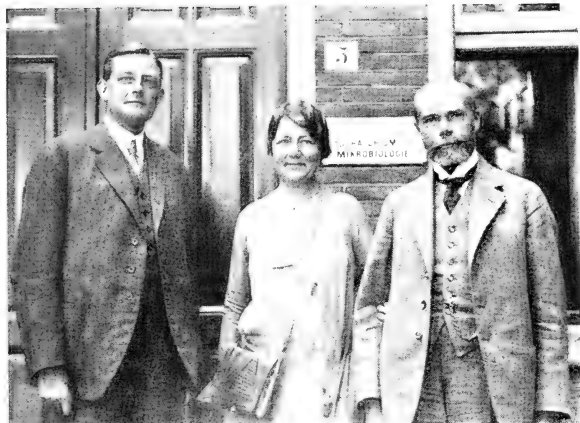
of the colour was

greenish yellow



3. A manuscript page. J. Bact. 72, 406. 1956.
4. The laboratory in winter.
5. A commemoration at the laboratory; 1933.





6. Orla-Jensen visiting the laboratory; 1929.

7. Kluyver and a student; 1937.

8. An excursion; 1938.

9. Kluyver and Van Niel; 1955.



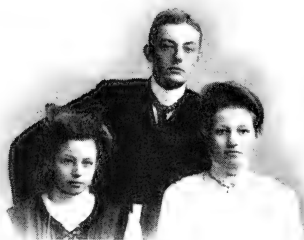


10. Promoter Kluyver at the promotion ceremony of Prince Bernhard of the Netherlands: 1931

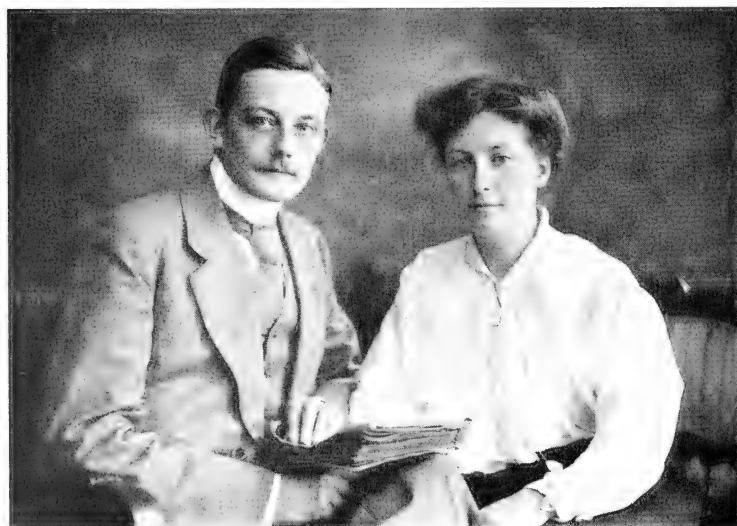
11. Prince Bernhard, Dr. Techn. Sci. h.c., and Kluyver after the promotion: 1931.

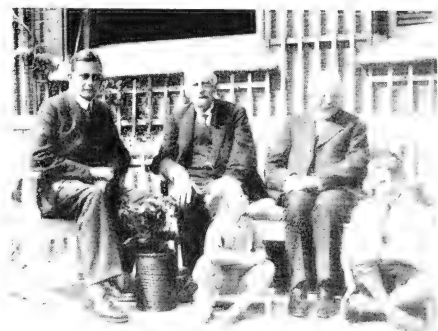
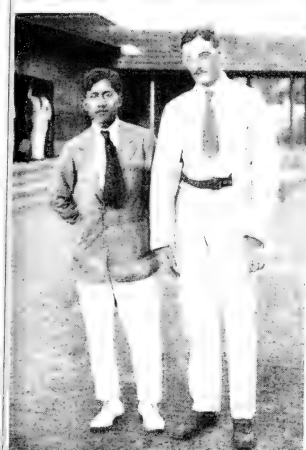
12. Kluyver as Rector Magnificus at the Student Union: 1938.





13. Kluyver as a youth; 1898.
14. Kluyver with two of his sisters; 1908.
15. On the roof of the Student Union, Sociëteit Phoenix; 1903.
16. Kluyver with his fiancée; 1916.
17. Kluyver and Raden Mas Iso Reksohadiprodjo; 1920.
18. The young Kluyver family in the tropics; 1919.
19. Holidays with his family in the Biesbos; 1933.
20. Three generations of the Kluyver family; 1930.
21. Another three generations of the Kluyver family; 1950.







22. Kluiver in his study; 1955.

KLUYVER AS SEEN BY HIS PUPILS

'He is great who is what he is from nature, and who never reminds us of others. But he must be related to us, and our life receive from him some promise of explanation. I cannot tell what I would know; but I have observed there are persons who, in their character and actions, answer questions which I have not skill to put.'

(R. W. Emerson)

To students at the Technological University in Delft the Laboratory of Microbiology used to be known as a quaint old building at the Nieuwelaan, bordering on the canal and adjoining the premises of the student's Rowing Club; and with most of them knowledge went no further. To the chemistry students it was also one of the laboratories where they might choose to spend their last year before graduating with the Chemical Engineer's degree. The number of these latter who elect microbiology as their field of specialization has always been relatively small, however; students who are interested in things biological are naturally more apt to enroll in other universities where biology, instead of being merely an adjunct to the chemistry department, has its own rightful place in the curriculum.

The Delft students who have qualified as candidates for the Chem. E. degree and enter the microbiological institute have had a rigorous training in all branches of chemistry, in physics, and in mathematics; but they have no more than a faint inkling of the properties of animate matter. There can be no doubt that their choice has usually been influenced by a more or less conscious dissatisfaction with the inflexibility of the behaviour of molecules on the one hand, and on the other by the intriguing opportunity to study a new subject that will expose them to the alluring mysteries of life itself.

It is precisely to this frame of mind that the mentality of Kluyver and the atmosphere of his institute were most receptive. Kluyver himself had probably passed through a similar development during his studies at the Delft university; as a candidate for the Chem. E. degree he had majored in microscopical anatomy, and later he had continued

to prepare a doctor's thesis on a biological subject under the guidance of Van Iterson. Following his appointment to the chair of microbiology, he had been compelled to master this field by himself, and this, too, must have tended to a certain feeling of kinship with his students. During his student days in Delft he had maintained a close contact with friends at the University of Leiden, which had stimulated his interest in biology and widened the scope of his knowledge. Nevertheless, he was proud to be an alumnus of Delft, and thus eminently suited to initiate his students in a field for which their previous training had hardly prepared them.

Thus the microbiology novice was at once received into a congenial atmosphere that had been created by the Director and pervaded the entire laboratory. Its most striking feature was the spirit of complete freedom; there were no rules and regulations; it was taken for granted that everybody was enthusiastic, did his best, and behaved as a mature individual, showing responsibility towards the books, the stores, the equipment, and other workers. There were no formal restrictions; in principle everything was and should be possible; if there were obstacles, every effort was made to remove them. The only limiting factors that were recognized were the laws of nature, illness being grudgingly included among the latter. The lab was never really closed. The work was carried out in a true holiday spirit, and the Director's response to a question about vacations, put by a visiting scientist, was as simple and natural as it was exact: 'My dear Sir, here every day is a holiday'.

The building itself, once described by a visiting journalist as a cross between a hunting lodge and a sanatorium, was designed throughout on functional principles to the point of barrenness; every nook and cranny was exploited and adapted to some special use; and the frequent changes, dictated by the needs of the moment, endowed it with a spirit of intensive and efficient internal life. This, together with the odd architecture, managed to create a romantic impression which was further enhanced by the appearance of the eminently practical library rooms, and which reached its climax in the study of the professor, the 'Olympos', the centre of all things around which the laboratory and the house had been built.

That large room had three windows, all facing south with a fine view of the canal. The remaining wall space had been used for the storage of books, reprints, and all kinds of documents. There were also

many photographs of friends, pupils, and colleagues, as well as curios and mementos, such as a replica of a Leeuwenhoek microscope, a stuffed kingfisher, and an Iowa State College pennant that often aroused the curiosity of visitors. The room had two doors, one leading to the house, the other to the laboratory. It contained the desk and chair that had belonged to Beijerinck, and it was said that during the first several years Kluyver could hardly overcome his awe, and did not dare use them. A weird assortment of four easy chairs around a small table in a corner of the room, and a huge table with six beautiful, antique, straight-backed chairs around it completed the furniture. It was in the chair at the head of this table that Kluyver did most of his work; when his collaborators were in the study, they installed themselves to his left and right. Visitors were accommodated in the 'cosy nook', offered cigars and cigarettes, and interrogated shrewdly, yet so courteously, that they hardly noticed how much they revealed. Every interview was afterwards recorded on a separate sheet of note paper in a few highly pregnant words or sentences.

All the drawers bulged with papers, especially in later years. Kluyver received innumerable reports and reprints, and because most of them contained something of interest to him, he could hardly ever bring himself to discard a single one. They were therefore stored in his 'bar of science', a specially constructed set of shelves, from which he could always extract the most diverse information pertinent to a particular discussion.

The over-all impression of the room was neither one of beauty or academic, awe-inspiring austerity, nor of bohemian carelessness. Its quality was in harmony with its occupant; each object, by its nature and place, possessed a vital and graphic significance. There was nothing to hide, and hence it revealed, honestly and unpretentiously, what its owner did and how he did it. It was a workshop that never failed to bring the visitor under its spell, radiating adventure and accomplishment.

No wonder that many of the students felt an instantaneous affinity when visiting the director in his room. This did not occur frequently, however; most discussions with the students took place at the workbench in the laboratory or in the library, the domains of the students and of experiment. The formal guidance they received was negligible, and consisted mostly in the tacitly implied conclusions that could be

drawn from the discussion. Explicit advice or instructions were not given, for Kluyster knew that such direct help does not exert a lasting influence. He strongly felt that the initiative should come from the student; that a solution found by one's own efforts is of immeasurably greater value than one given by another person. The discussions with the students were informal; seated on one of the low stools Kluyster examined their cultures with a small magnifying glass or under their microscopes, patiently letting himself be informed about recent developments, putting in a question here and there to get a detail straight, and asking for suggestions as if he were the pupil. The next morning the student might find on his desk some reprints that Kluyster considered pertinent; but it happened more often that during the conversation professor and student would migrate to the library where, as the search for information went on, pyramids of books and journals would soon accumulate on the tables. In the course of these explorations, usually attended by one of the assistants, the facts and arguments for and against certain interpretations were clearly developed, and thereafter the student was left to his own devices. Kluyster always groped for the essential aspects of a situation, and, like Beijerinck, insisted that an experiment should be simple; simplicity was to him also the best criterion of the merit of an interpretation or theory. Kluyster never used his authority and great experience to drive his pupils in a particular direction, though he tacitly assumed that, as rational beings, they would set their further course by the outcome of these discussions. He delighted in every spark of original thought or experiment. If the students deviated from the approach he had hinted at, he made sure that they were aware of the warning beacons, and then let them proceed at their own peril. Even in cases of extreme stubbornness his exasperation was evident only in private conversations with his associates, and he refused to interfere. 'We must have patience. Mr. X has to work out his own salvation; we cannot hand it to him on a platter'.

The curriculum requirements included specified minimum periods of laboratory work, satisfactorily completed. Kluyster did not like to be the sole judge of what was 'satisfactory', and argued that the main point at issue in determining whether the student had done enough was the latter's own opinion; 'his soul had to be satisfied'. He saw no point in keeping students any longer than they themselves considered necessary; those who wanted to stay on were, of course, always wel-

come to do so. They ought to be wise enough to recognize their task, and otherwise to ask for guidance. They were expected to do as much as lay within their capacity.

A natural corollary of this attitude towards the student was that those who preferred working under concrete orders in a definite schedule had difficulties in adjusting themselves. Some of them never did, and eventually left, disappointed. Kluyver was fully cognizant of this, but it did not make him spoil his game. He was tolerant of defects in scientific education and even in character, provided some enthusiasm and a positive will were evident; but these he neither could nor would supply if his own example failed to engender them. He accepted the fact that, after all, not every student can be sufficiently interested in microbiology to make him devote his major efforts to its pursuit, and he did not respect such persons any the less for it.

In this manner Kluyver established an ideal relationship with his students in a very short time. It is hardly surprising that, once they had started to work in his laboratory, they more often than not felt as if they had landed in heaven. Their expectations were realized more completely than they could have dared to hope; for, with the intuition of youth, most of them quickly recognized the greatness of their teacher. This made it easy for them to acquire a satisfactory knowledge of the elements of microbiological science. Usually the students had already taken Kluyver's introductory laboratory course in general microbiology in which were included numerous enrichment cultures and pure culture isolations. Moreover, they could attend two series of lecture courses, one in general microbiology, the other dealing with one of four special topics that alternated from year to year. In his lectures Kluyver used an inductive approach; from a gradually accumulating body of experimental facts fundamental concepts were evolved. Every lecture was illustrated with an abundance of demonstration material. The greatest care was lavished on the first few lectures of the general microbiology course; for it was here that the sceptical young chemist was first formally initiated into the mysteries of living organisms. Here, the demonstrations included the scrupulously repeated classical experiments of Leeuwenhoek and Pasteur; and their significance, coupled with their simplicity and surprisingly primitive equipment, produced an imposing, nay, even solemn, and somewhat romantic effect.

A candidate for the Chem. E. degree was required to submit a detailed report of the research he had conducted during his final year, and this report had to be approved by the major professor. Kluyver discussed these reports with his own students punctiliously, from beginning to end, sometimes spending more than a full day on a single one. A few days later, professor and candidate met again for the official examination ceremony where, in the presence of the entire faculty of the chemical technology department, the candidate was subjected to an interrogation of some ten minutes' duration by his major professor. On the whole, this examination was a mere formality; but it was characteristic of Kluyver that he always succeeded in making a sporting event of it by subtly creating the impression that the candidate had to fight for a passing grade. The result was invariably gratifying; the ceremony gained in gravity, and afterwards the student could look back upon the performance with far more satisfaction than if he had been convinced that it was something of a farce.

The degree earned, the new Chem. E. had to look for a job. Kluyver realized that a microbiological education limited to a single year did not suffice to make anyone a full-fledged microbiologist, and he did his utmost to help those who wanted to extend their training and experience by continuing to work in his laboratory. On the other hand, since Kluyver was regularly consulted by various industries, he often acted as an intermediary when job opportunities presented themselves. For the students it was but natural to seek the advice of the master when confronted with such problems; but they never obtained a direct answer. Kluyver would survey the available positions and possible careers, interspersed with questions about the inclinations of the person in question. The latter would afterwards go home with his ideas sorted out and his mind already half-way made up. Kluyver seldom went so far as to invite a promising student to stay on as an assistant; he clearly separated the interests of his students from those of the institute; and, even if they declared their preference for continuing to do academic work, he first called attention to various possibilities here and abroad, only at the end mentioning that a position might perhaps be found in his own laboratory. Then he would dwell in detail on the drawbacks of such a solution, and only hint at the positive aspects. But if the young graduate, after such 'fair warning', still insisted on his preference for an assistantship in the Delft institute,

Kluyver saw to it that this signified the start of a greatly intensified education.

The first duty of the new assistant was usually the preparation of the demonstration material for the lecture courses. He could lean on an old, hand-written manual, compiled and amended by successive generations of assistants, for planning the execution of the experiments which were required for a particular lecture and which had been outlined during the special weekly conferences with the professor. Nevertheless, the task made heavy demands on the time and capacities of the young assistant. Every week new experiments were needed and even though, aided by the information contained in the manual, he managed to get them started with a minimum of delay, some of them were not immediately successful. The limited time available, with the deadline determined by the time of the lecture, did not always permit of repetitions, so that at the critical moment the demonstration material might expose more or less serious gaps, a permanent source of worry for the assistant. It was Kluyver's custom to inspect the results just before the lecture, accompanied by the anxious assistant; he never complained about the bad experiments but singled out for praise those that had come out well; and he could make the assistant feel that the whole success of the lecture actually depended on the latter's contribution. One may, of course, question the efficacy of demonstrations as far as the education of an audience is concerned, and Kluyver himself certainly did not overestimate it. His main reason for perpetuating the practice was its immense value in terms of the education of the assistant who had a unique opportunity to become familiar with a wide variety of organisms and broaden his practical experience under expert guidance. Another important reason was that these experiments led to the accumulation of valuable information, covering a wide range of subjects; occasionally they cast doubt on current views, and then yielded problems for more detailed studies afterwards.

In addition to the above task the assistant usually conducted experiments on a subject of his own choosing, which had been agreed upon during extensive deliberations in which both the assistant and the professor voiced their ideas. It is not surprising that the great freedom Kluyver allowed his students implied that often a great diversity of problems was being simultaneously investigated in his laboratory. Although this widened the horizon, it may have impeded the rapid de-

velopment of any one specific topic. Again it is clear that Kluyver did not close his eyes to this aspect of his policy; but he consciously did not encourage teamwork during the formative years. He preferred the cultivation of the single individual, who, thrown back on his own resources, could thus engage in a study for which he had evinced enthusiasm from the start.

The same type of guidance was received during the work for the thesis; here too the burden of initiative rested with the candidate, the professor acting the part of the obstinate pupil who had to be convinced. When a manuscript had finally been prepared, it was scrutinized in the most meticulous fashion; every sentence, every word, was weighed and criticized with unassailable reasoning, leading to revisions in text and in the arrangement of data. An excellent impression of this process may be gained from the following description of Kingma Boltjes, which applies to the humblest publication and the most elaborate thesis alike.

‘During lengthy evening sessions, often extending till deep into the night, the document is critically discussed. Subsequently, Kluyver literally makes an armed attack on the manuscript, while the author, according to his disposition, resists to a greater or lesser extent. But the end result is invariably the same. With a large pair of scissors the product is ruthlessly, though in a carefully considered manner, cut into pieces. The parts are moved about, and much has to be rewritten in order properly to fit the original pieces together into the new pattern. Undeniably, the construction thus becomes more logical, and particularly the readability is immensely improved. An attentive reader will readily recognize the publications prepared under Kluyver’s guidance by virtue of the numerous distinctive phrases they contain; Kluyver himself refers to them as “Leitfossilien” or graptolites. During this undertaking no trouble is too great for him; quietly, unperturbedly, unaffected by time limits, the entire manuscript, down to the last comma, is examined so to speak under a magnifying glass, with the justification that “Genius is the infinite capacity for taking pains”. When at last the proofs arrive, Kluyver actively participates in their correction, and he thinks nothing of assisting with them till late in the night. Astounding in this connexion is Kluyver’s indefatigableness. Whereas, by the time of the finish, the writer of a dissertation is visibly worn out, Kluyver remains cool and collected to the

very end. Only if a number of theses are written in quick succession is it possible that he may show some signs of fatigue; but these he overcomes in an amazingly short time.'

With advancing seniority the assistants gradually became more involved in the organizational work of the laboratory and acted as aides to the director in many different spheres. Thus they had an opportunity to observe, behind the scenes as it were, how he worked and handled people.

Kluyver's working days always started with a perusal of the mail. All 'easy letters' were disposed of right away, in later years the answer being dictated to his secretary. Some letters might fall into the category of 'procurement of labour', and these were put on one of the piles on the table, according to urgency and interest. Proofs were immediately corrected and returned. The receipt of reprints and other publications was generally acknowledged in a personal note that always contained some cheering and pleasing remarks, in tune with the personality of the addressee. It was hard for Kluyver to use the same text twice, and he detested printed or mimeographed forms.

He was always ready to help whenever his assistance was solicited. On the other hand, he was disinclined to beg favours from others, to 'make a bow', as he called it, unless it was absolutely necessary. Then his letter made it clear that a favour was being asked, and that there was no obligation whatever to fulfil his request, for this was the way in which he himself liked to be treated; as he had learned, it minimized the chances of getting into trouble. No one could ever claim the right to the cooperation of others, and one should be duly grateful if it was granted. All in all, his attitude was reminiscent of the nobleman's courtesy.

He often referred letters to one of his associates for an opinion or to have some details checked before answering them. Now and then such letters indicated the author's incompetence, or a distressing kind of reasoning. But Kluyver never allowed censorious or deprecatory remarks to be made on such occasions; disposing of the clumsy aspects of the letter in some poignant statements, he would proceed to explain the material difficulties under which the writer worked, and emphasize his attainments in other respects.

Sometimes inquiries were made about details of investigations still in progress, by people working on the same subject. In such cases

Kluyver's first reaction was always to cite the memorable words of Cornelis Tromp, the Dutch admiral who had proposed to send powder and ammunition to his Spanish adversary in order to insure that the battle might go on without an insuperable handicap on one side. Or, to cheer up the associate in question, he would cite the words of Kipling: 'They copied all they could follow, but they could not copy my mind, and I left them swearing and stealing a year and a half behind.' But soon he would face the interests of his own pupils, and the end result was usually a satisfactory compromise.

It was a delight to be in attendance when Kluyver was writing a letter or, for that matter, to witness the process of a well-considered argument taking shape in any manuscript from his hand. He used a vocabulary and style that were unique, and had developed, in Dutch as well as in other languages, a distinctive Kluyverian idiom which was apparent also in his speech. It was based on the premise that the meaning or effect aimed at should be inescapably clear, and expressed in phrases of irreproachable grammatical correctness. At the start a *captatio benevolentiae*, a bow to the reader, was needed to catch his attention; at the end a climax to drive the point home. Within this framework Kluyver took advantage of every opportunity to insert his typical, flourishing expressions, usually overstatements, the fruits of quick wit and rich imagination, designed to amplify his intentions with sudden gushes of surprising perspicacity. Not infrequently he spiced his speech and letters with veiled and highly subtle criticisms that were not always immediately grasped, and thus had the effect of a time bomb, producing an unexpected shock when later their true significance began to dawn upon the recipient. Instead of criticizing people to their faces, with the prospect of jeopardizing pleasant relationships, he only supplied the pieces of a jigsaw puzzle from which the listener could afterwards reconstruct the true state of affairs in strict privacy; this eliminated the need for referring to it on later occasions. Praise and encouragement, too, were often wrapped up in the same way, and for the same reason. He liked to tease a little those who could stand it, including an audience or his readers, in order to dispel even the semblance of pomposity. Thus he evoked reactions that could not have been elicited as easily by other means. It has been noticed that many of his pupils unconsciously picked up some of the ways of their master, and could thus be unambiguously identified as such.

He took great pains with his texts; his mastery of languages was impressive. Although he had never learned Greek or Latin, nobody would have suspected this. Among foreign modern languages he seemed to have a preference for English; and armed with Roget's 'Thesaurus' and the Oxford and Ten Bruggencate's Dutch-English dictionaries, he liked to hunt for the exact word. He had a predilection for words that appealed to him as expressive, and an aversion to weak ones, such as 'interesting' which, he used to say, 'ought to be used only by one's aunt in reporting on a lecture she had not understood'. Van Niel was a match for him in linguistic matters, and their collaboration on the text of the Prather lectures was a delight to both.

Although Kluyster was not the ivory-tower type of scientist, he allowed his social contacts and family life to usurp as little of his time as was reasonably possible. His wife, bearing the brunt of leading the household and of educating the five children, nobly and unselfishly shielded him from most cares. Kluyster did not like to bother with matters that seemed to him trivial, including the greater part of his personal affairs; and when his decision on some household transaction could not be dispensed with, Mrs. Kluyster frequently had to come to the study and compete for his attention with his collaborators. If, on such an occasion, Kluyster's interest had been kindled and he started to analyse the matter in the same expansive manner in which he approached a scientific problem, she was quick to pierce his eloquence in her businesslike, matter-of-fact way with some such ejaculation as 'Don't show off, Ab!', thus saving her time and his; and Kluyster graciously conceded the common sense of this attitude. Her vigour, cordiality, and efficiency enabled her to cope easily with meals or garden parties for any number of guests who often turned up quite unexpectedly. We may be sure that Kluyster did not take this essential support for granted or that he accepted it as his due; he was slightly apologetic about it, with an ever fresh sense of gratefulness.

Thus Kluyster could devote virtually all his time and energy to his various tasks. The day was divided into three parts, starting at 9 a.m., 2 p.m., and 8 p.m., respectively, each one terminating at rather indefinite times. Only in later years did he allow himself a short break during the morning when, at 10.30, superb black coffee, much coveted by his associates, was served in the study. Kluyster adhered to his schedule with great punctuality, though never demonstratively so. He

really felt unhappy and guilty if he did not work in the evening, perhaps out of a sense of duty left over from the early years when he had to spend all his time studying in order properly to prepare himself for teaching a science that was new to him. No doubt this attitude was intensified by the feeling that he had incurred the obligation to strive towards attaining a level of excellence such as Beijerinck had also reached by working incessantly.

Kluyver had no ear for music, though he listened occasionally to an opera on the radio, gaily humming along. Apart from his interest in tennis and major sports events, he had no 'worldly hobbies' to which he could turn for relaxation. His only ruling passion was for work, 'bound by neither time nor eternity', and aided by his enormous reservoir of physical strength and power of recuperation. He never pampered himself; and, although appreciative of the good things of this earth, he would not go out of his way to acquire them, and generally adhered to an austere regime. It was only a few months before his death that he confessed to having taken the first after-dinner nap in his life; 'the colloquium had been so tedious and complicated' was his apology. He was quite susceptible to the charms of travel. His visit to Rome in connexion with the 6th International Congress for Microbiology had filled him with such admiration and love for the Eternal City that he allowed himself the uncommon luxury of going to the cinema to see 'Roman Holiday'; a strong additional motive was the fact that the leading actress was of Dutch descent. Nonetheless, it would never occur to him to travel for its own sake. If he went on a short vacation at all, he usually stayed in Holland, most often in the country near Kootwijk, where there was ample opportunity for walks in the woods, hunting mushrooms and picking blueberries with the children. From the small things in life – a beautiful morning, a subtle joke shared, the first drawings of his granddaughter, Eja, which he proudly displayed in his study – he derived a keen and genuine pleasure.

What was the end to which Kluyver dedicated practically his entire life? It is certain that the pursuit of microbiology cannot be the only answer. Of necessity, during the first decade of his professorship this had to be his major occupation; but as soon as his accomplishments permitted, he branched out and attempted to relate his field to others, giving scope to his wide interests and desire for integration. Con-

sequently his membership in the Royal Netherlands' Academy of Sciences, where each month he could meet with the outstanding representatives of many branches of the natural sciences, was to him far more than an honour: it provided him with the means of satisfying his real need for a better comprehension of nature and of man. Though he may not have been conversant with the methodological details in other fields, he nevertheless kept abreast of developments in biology, physics, astronomy, geology, and medicine. The extensive knowledge so acquired is reflected in many of his publications, and strikingly apparent from the obituary notices of Academy members which he wrote during his tenure of the presidency of this body. In addition he was a member of a whole spectrum of learned societies, committees, and editorial boards, thus encompassing a much broader field than microbiology proper.

Although he was a scientist first and foremost, he did not shirk other duties resulting from his professorship, and he did not confine himself to those connected with his laboratory, faculty, or even university. He was deeply interested in the functional aspects of higher education in the Netherlands, and on many occasions, *e.g.* during his rectorate, his special gifts were naturally put to good use.

Apart from contributing to the advancement, organization, and dissemination of science, he felt it his duty and prerogative as a scientist to keep his finger on the pulse of the development of mankind in the broadest sense. He wanted to be informed about current events in the world at large, and to gain a reasonable interpretation of their meaning. To this end he read widely and voraciously, and subscribed to numerous Dutch and foreign journals, including the 'New York Herald Tribune', 'Time Magazine', 'Biology and Human Affairs', and 'Impact of Science on Humanity'. Foreign policy fascinated him, and was often discussed with his associates. He had files of newspaper clippings on many matters of general interest, such as the Salk vaccine, and did not consider it beneath his dignity to peruse scientific reports written for the layman, particularly because these often suggested novel ways of presentation. Such an interest is comprehensible because he himself had a strong and efficacious inclination towards colourful display, adapted to the audience of the moment, as is evident from many of the slides and charts he had designed.

Against this background it is understandable that the second world

war and occupation of his country had a terrific impact on Kluyver; in a situation where logic and good intentions were superseded by brute force and malice he could not feel at ease.

The real and potential disasters of the war took full possession of his keen mind and sensitive heart; they displaced many things that had previously occupied the forefront of his attention. Kluyver's attitude towards science and human affairs that was so characteristic of his later years must have been conditioned during this time by the need for a reportioning and reappraisal of values, opinions, and persons. The war intensified his preoccupation with both the larger issues and the individual. He had even fewer illusions, was more humble and warm-hearted, but at the same time more powerfully outspoken after the experience.

Especially during the post-war years Kluyver displayed the qualities of a modern universal scientist who remained in close touch with the outside world. This exacted demands that are colossal in comparison with those required of the medieval scholar who had ample opportunity to pursue his own researches and at the same time to give his pupils extensive and profound instruction by merely letting them look over his shoulder. The social position and responsibility of the scientist, the tendency towards exponential development of research, and the dramatic expansion of man's technological resources, these were matters to which Kluyver devoted much of his time and energy, and with which he also confronted his collaborators. To the end he kept on learning, and he continued to teach his beloved specialty, microbiology, in a manner that emphasized more and more its place in the scheme of science and the implications of science for mankind. Free from the narrowness and irresponsibility with which the scientific profession is so often stigmatized nowadays, he restored to it dignity and humanity, and set an example for contemporary and future scientists.

We cannot say that Kluyver was compelled by an absorbing passion for truth, any more than that he was primarily motivated by the urge to promote the welfare of mankind. He worked because he liked it, and he was ever grateful for the opportunity given him. In later years he even felt apologetic about the position he occupied, because he thereby prevented a younger man from enjoying and exploiting its wealth of possibilities. He may have thought of his own activities as

pleasant and acceptable occupations, befitting the gentleman he was. Had he chosen to do so, he might have excelled in any one of a number of fields quite remote from microbiology.

His self-respect made it impossible for him to set any standards save the highest, the only challenge worthy of his endowments. There was no point and no fun in doing something if it were not done as well as possible. Once engaged in a task, he never backed out; his pride allowed of no defeat. And in this manner he cultivated also in others a strong sense of responsibility and loyalty.

His keen and critical intellect, sustained by reasonableness and a sense of proportion, made him the type of 'classical scientist' whom he characterized in his Washington speech. But there was more. Perhaps the gypsy vein, said to run in his family, expressed itself in the undeniably adventurous and romantic traits of his character, and most of all in his intuition and infinitely sensitive receptiveness to human emotion. These elements, unified into a harmonious bipolarity, conferred upon him an uncanny perceptivity through which he experienced reality in all its complexities. In both directions his vision extended all the way to the horizon, and the ominous comprehension of endless possibilities could not but produce an attitude of wariness and uncertainty. The need to make decisions was to him one of the crude necessities of life, and a decision once made always remained tentative and subject to an 'agonising reappraisal'.

But living with insecurity did not depress him, nor did it make him cynical. He had accepted it as inevitable, and learned to master vulnerability without becoming immune to it, by adopting a charming formality of manner that nonetheless made it easy to approach him. Through his courage and strength of mind he could keep his balance and act in practical life without having to distort the labyrinth of doubts into a more reassuring though false pattern of 'truths'.

He had no interest in seeking objective perfection – perhaps he was simply bored by it – but rather strove towards finding the best possible solution for a given situation in which the pertinent factors, human as well as others, had been taken into account. His own work, conclusions, and decisions bore in his mind a predominantly conditional and experimental character, particularly because they had been framed in a mood bordering on playfulness; to others they may have appeared as the last word and unassailable truths.

Every scientist knows, or should know, moments of doubt towards his own achievements. It is clear that Kluyver, who perpetually lived with doubt, so to speak, had become an expert in handling those sets of incomplete and obscure data that Nature is wont grudgingly to grant the biologist. He realized as few others that in science it is necessary to focus attention on some particular aspect, abstracted from its surroundings. He was a master in the art of emphasizing the crucial ones amongst a multitude of facts, and of distinguishing between scientific risks and reasonable interpretations. No wonder that many scientists, who instinctively turn to their neighbours for recognition and assurance, looked to him for appreciation of their work and restoration of their peace of mind. To them he was a yardstick of achievement; for science is but a frail network of concepts, kept intact by the courageous few who can substitute guiding principles for certitude. And Kluyver was one of them.

Of course, his science and profession were constant victims of Kluyver's pragmatic scrutiny, and his sense of humour did not stop at his own person. His self-respect could not be founded on the awe in which laymen may hold the scientist, nor on the esteem of his colleagues, nor even on the recognition of participating in the improvement of the conditions for man's existence. He was not deluded by the facile fallacy of mistaking results for aims. A clue to Kluyver's attitude may be found in a passage of the speech he delivered at a commemoration ceremony for alumni of the Delft university who had perished during the war. Here he groped for the motives that had induced these young people willingly to risk their life in the resistance movement. Vigorously denying that love for their country could have been their main incentive, he ventured to suggest that they had so acted because 'C'était plus fort que moi'. Similarly, in his lecture entitled 'Homo militans' he called attention to the simple words, 'Par honnêteté', in which Camus formulates the answer to a comparable question in his book, 'La Peste'.

The essence of Kluyver's personality was revealed in his relations to people. He had a wonderful propensity for spurring others on to higher attainments; by emotionally stimulating and coolly criticizing them, he carefully prepared the conditions under which they could develop to the best of their potentialities. He directed by soliciting ad-

vice; he taught by asking questions; he armed himself by refraining from fighting. With just that little more patience than the average, he could manoeuvre himself into the strongest position, and usually managed to accomplish what he wanted. He hardly ever worked in the laboratory with his own hands; he used people as well as his pen as his instruments.

Indubitably these great talents could have assured Kluyver of greater and more spectacular successes had he cared to exercise them to these ends. We can only conclude that he had diverted his ambitions from the common path and held uncommon personal views as to what is significant.

Turning to Kluyver's attainments, we may pass over the more obvious ones in the field of science, and concentrate on the less tangible effects of his labours. The profound influence he exerted on those around him has been stressed even in articles on Kluyver as a scientist. It was indeed a great and intense experience to be exposed to the radiance of his personality. He had acquired the rare ability to transfer effectively the fruits of his experience to other people, a quality perhaps greater than wisdom. He had known the sting of ambition, the tensions of failure and success, of fear and inferiority; but he had come through unscathed, soaring above them with a smile. He had related his knowledge of the 'infinitely small' to life as a whole and to man's place in it. His teaching and research had grown from an end into a means; and by communicating his wisdom to his associates so that it always filled their individual needs, he made a lasting contribution to their education. With the rich expressiveness of his face and subtle speech he could uncover unsuspected potentialities in his pupils, and convey enduring aid and comfort. In this way he also transmitted moral codes that were almost unconsciously adopted by recognition.

This powerful influence of Kluyver on his fellowmen has been touchingly described by Senez, who, after Kluyver's death, wrote: 'J'ai fait à Delft, il y a déjà plusieurs années, un trop court séjour, dont je garde un souvenir très vif et très précieux. Votre Maître, Monsieur le Professeur Kluyver, m'y avait reçu avec cette simplicité charmante qui était la sienne et les quelques jours passés auprès de lui ont très profondément orienté ma carrière scientifique. Son rayonnement était immense et il existe dans le monde entier des chercheurs qui se con-

sidèrent comme ses élèves. Permettez moi de me ranger dans leur nombre et de vous dire combien je suis attaché à sa mémoire.'

Another manifestation of Kluyver's concern for individuals, characteristically cloaked in practical terms, may be found in his after-dinner speech in Washington on 'An Aspect of the Promotion of Science'. Here he made an eloquent plea for the 'romantic scientist', the genius who has difficulties in adjusting himself to society. On the ground that 'we cannot afford to let scientific geniuses perish', he advocated the establishment of institutions where these men could work under conditions attuned to their particular needs. Those who have known Kluyver will readily infer that this plea was not based on the stated motive alone, and that he was also, perhaps even chiefly, moved by a direct solicitude for these geniuses themselves. Practicing more than he preached, he extended these ideas to the humblest of his pupils, providing for them an environment where they could thrive and develop to the best of their abilities.

This concern for others dominated all his actions, harmoniously imbuing them with added significance in purpose and effect. The response must have enriched his life, and may perhaps have compensated for the utter loneliness that his position inexorably engendered.

In any group, whatever its function, he saw furthest, and thus irrevocably became its focal point. It was not for him to be comforted; he could not afford to let responsibility slip away or to indulge in the easy escape into anger.

His perception of the infinite potentialities of life had made him recognize the human mind as the acme of evolutionary development. Hence he believed that man's particular role in future evolution should rest upon the proper exercise of his spiritual powers. Only a few days before his death he attested to this conviction by quoting a fragment of the words of the Dutch poet, A. Roland Holst, engraved upon the National War Memorial in Amsterdam that had recently been unveiled:

'Never, from ore to eagle, was any creature free beneath the sun, nor was the sun, nor were the stars. But spirit broke law, and in that breach uplifted man'.

Thus, above all else, Kluyver was a man who moved in life's true

domain, to which science is subordinated, and where success is not measured. And by doing so, he has imparted imperishable gifts to all those who had the great good fortune to cross his path. That he was revered by his pupils and associates is evident; and the reason may be summarized by another quotation from Emerson:

‘This is the key to the power of the greatest men –
their spirit diffuses itself’.

J. W. M. la R.

KLUYVER'S CONTRIBUTIONS TO MICROBIOLOGY AND BIOCHEMISTRY

'When Coleridge tried to define beauty, he returned always to one deep thought; beauty, he said, is "unity in variety". Science is nothing else than the search to discover unity in the wild variety of nature – or more exactly, in the variety of our experience.'

(*J. Bronowski*).

INTRODUCTION

THE profound and inspiring analysis of the meaning of science from which the above passage has been quoted would have been readily subscribed to by Kluyver, whose scientific contributions offer a notable example of a perpetual search in that very sense. How successful this search has been is strikingly apparent from a comparison of the status of biochemical understanding in 1922, when Kluyver entered upon his career as a microbiologist, with that of to-day.

In the second of a series of lectures delivered at Harvard University in 1954, Kluyver, after having reviewed the current ideas on biochemical reaction mechanisms, could state with full justification:

'In concluding I should like to give my opinion that, mainly owing to its impressive metabolic diversity, the microbe has made a major contribution to our general insight into the essence of metabolism. There can be no doubt that studies on microbial metabolism have directly fertilized similar studies on animal metabolism in many ways' [Kluyver and Van Niel, 1956, p. 72].

Nor can there be any doubt that the chief impetus to this development in our understanding of the essence of metabolism has been the enunciation of the concepts of the 'unity in biochemistry' and 'comparative biochemistry', both of them based upon the principle of hydrogen transfer as the common and fundamental feature of all metabolic processes. And it is hardly necessary to mention that these are the most far-reaching among the many contributions that science owes to A. J. Kluyver, even though his pertinent publications are but

rarely cited any longer. This, I believe, should be ascribed to the fact that they have been so completely assimilated that it has become superfluous to reiterate the basis on which present-day biochemistry is founded, just as for over a century chemists have stopped referring to Lavoisier and Dalton except in papers dealing with the history of their science.

When, in 1922, Kluyster officially assumed his duties as Professor of general and applied microbiology at the Technological University of Delft, Holland, the knowledge of the chemical activities of micro-organisms was virtually restricted to an awareness of a large number of more or less specific transformations that can be brought about by the diverse and numerous representative types. Except in a few institutions the study of biochemistry itself appeared to consist in little more than the development and application of methods for the analysis of urine and blood. Within ten years Kluyster succeeded in welding together a vast amount of detailed information into a coordinated picture, whose strong and simple outlines encompassed the totality of the chemical manifestations of all living organisms, and whose structure brought into strong relief the dynamic aspects of these processes. In another ten years the direction of biochemical research throughout the world had been guided into the paths mapped out by Kluyster. And the spectacular successes scored in enlarging and intensifying biochemical understanding through investigations with appropriate micro-organisms, an approach repeatedly practiced and advocated by Kluyster, had convinced an increasing number of biochemists of the potentialities of such studies, with the logical result that the post-1940 biochemical literature has become predominantly occupied by publications on various aspects of microbiological chemistry. These transformations attest to the great influence exerted by Kluyster's contributions; verily, it is difficult to overestimate their importance!

It is the purpose of this essay to trace the main stages of Kluyster's scientific development, and to indicate the wide area over which his fertile and philosophical mind was allowed to range during the 34 years of his professorship. It may then become evident that the many honours he received, and the great esteem in which he was held were richly deserved. But it must not be believed that his scientific eminence alone is responsible for the deep attachment that he engendered in his friends, collaborators, and pupils alike. For he also pos-

sessed many captivating and inspiring traits that were not directly associated with his scientific endeavour. These, however, fall outside the scope of this paper; nevertheless, those who have had the privilege of knowing the Master personally will not lightly forget how much they have benefited from their every encounter with him, and will often be reminded of the treasures they have been permitted to add to their experience.

THE PROLOGUE

In his inaugural address, reprinted in this volume, the newly appointed professor defended the thesis that some of the chemical activities of micro-organisms might be used to advantage on a commercial scale in order to supply the ever increasing need of an industrialized society for various raw materials. In this manner he could justify the inclusion of microbiology in the curriculum of the Technological University, and hence his own position. In accordance with precedent this public lecture also embodied some plans for a special research programme. But it must be admitted that these were extremely vague. This is not difficult to understand; at that time Kluyver was by no means an accomplished or experienced microbiologist. During his tenure of the position as assistant to Professor G. van Itersen Jr., from 1910 till 1916, he had, however, evinced a number of attributes that had made his appointment to the chair which Beijerinck had been compelled to vacate on account of having reached the age limit imposed by law, a sound 'calculated risk'. Some of these characteristics can be appreciated from an examination of Kluyver's [1914] major publication prior to that date, the thesis on 'Biochemical Sugar Determinations' which had earned him the degree of D. Sc. 'with distinction', the only manner in which the Technological University, on granting a degree, can express its recognition of superior merit.

With the aid of a simple apparatus, somewhat modified after a model originally designed by Van Itersen, he had demonstrated the feasibility of accurately determining the amount of carbon dioxide produced by yeasts during anaerobic incubation with sugar solutions. And, because yeasts can be differentiated in part on the basis of the particular sugars they can ferment, it seemed possible to use a number of judiciously selected yeast types for the quantitative determination of any one of a variety of sugars in mixtures of these substances.

An extensive study of the properties of a large number of yeast strains led to the selection of a limited group exhibiting the most desirable patterns of sugar fermentation. It included two new isolates, described in the thesis as *Torula monosa* and *T. dattila*, n. sp.; like the other members of the small collection both had been found to ferment rapidly and completely glucose, fructose, and mannose. But *T. monosa* appeared to be incapable of fermenting any other sugar, whereas *T. dattila* could also ferment sucrose. The additional strains were chosen because they could ferment one or more of the following sugars besides the above-mentioned ones: galactose, maltose, lactose, melibiose, and raffinose.

For each of these strains the quantitative relationships between carbon dioxide production and amount of fermentable sugar were next determined; thus it was established that there existed a linear proportionality between these quantities over a wide range of carbohydrate concentrations, and that the absolute value of the relationship showed slight but consistent differences between the individual cultures. In this manner the requisite calibration data were obtained; once these were available it was possible to compute the quantity of fermented sugar from the amount of carbon dioxide evolved. Consequently the quantity of glucose, fructose, and mannose combined could be estimated from the amount of carbon dioxide produced by *T. monosa*; sucrose from the extra carbon dioxide formed by *T. dattila*; maltose from that evolved by *Saccharomyces cerevisiae*; and other sugars in like manner from the results of fermentations with other yeasts.

During this investigation it was further established that the raffinose-fermenting yeasts can be divided into two categories; one group produces from the trisaccharide an amount of gas that corresponds closely with two molecules of carbon dioxide per molecule of raffinose, whereas the other liberates three times as much. The members of the former category, in contradistinction to those of the latter, were shown to be incapable of fermenting melibiose. Even closely related yeast types, such as bottom- and top-yeast, both classified as *S. cerevisiae*, can be readily distinguished on this basis; only the former ferments raffinose completely.

The simplicity of the method, coupled with its great specificity, made it eminently suitable for the purpose of quantitative determinations of individual sugars in mixtures. Kluyver specifically studied a

number of applications; he showed, for example, that it can be used to distinguish sharply between commercial jams and preserves prepared with the addition of sucrose, and of potato- or corn-sugar, respectively. This rests on the fact that the latter materials, produced by an enzymatic hydrolysis of starch, contain a high and reasonably uniform percentage of maltose, so that from the quantities of carbon dioxide produced by *T. monosa* (sucrose and maltose negative), *T. daktila* (sucrose positive, maltose negative), and *S. cerevisiae* (sucrose and maltose positive), respectively, the amounts of hexoses, sucrose, and maltose can be computed. A similar application was later developed by Den Dooren de Jong for the determination of lactose in bread; this permitted a ready detection of falsification of 'premium bread' which, according to the Dutch pure food laws, must be prepared with the addition of a specified minimum amount of milk to the dough. The constant lactose content of milk, together with the inability of baker's yeast to ferment this sugar, imply that the quantity of lactose in 'premium bread' is rigorously determined by the amount of milk added. The simple and specific estimation of lactose by the fermentation method thus provides an accurate check. It may be mentioned that in his thesis Kluyver had already hinted at the application of lactose-fermenting yeasts for the analysis of milk products.

Kluyver used his method also for the determination of the specific sugars present in glucosides; in leaves after periods of photosynthesis or storage in darkness; in germinating seeds and various other plant products; and in urine and blood. During these studies two unusual features of one particular yeast were discovered. The established production of carbon dioxide from pyruvic acid by yeasts led Kluyver to investigate the possibility that other acids, especially those generally encountered in fruits, might also yield carbon dioxide under the influence of certain yeasts. In these experiments it was found that *Schizosaccharomyces pombe* does, in fact, decompose malic acid under anaerobic conditions, provided a fermentable sugar is simultaneously present. The fermentation of malic acid can then be expressed by the equation:



In experiments on the fermentations in sugar-containing urine samples by various yeasts the observation was made that an initial carbon dioxide production was occasionally superseded by the eventually com-

plete disappearance of the gas. Pertinent tests revealed that this phenomenon, again encountered only with *Sch. pombe*, was associated with the development of an alkaline reaction in the fermented samples. The alkali production could then be traced to the hydrolysis of urea by the yeast. Thus it was ascertained that this species is unique among yeasts in that it can ferment malic acid and possesses a strong urease activity as well.

The numerous experiments carried out in preparation for the development of the method of biochemical sugar determinations had revealed several regularities in the fermentation patterns of different yeasts. These can be summarized as follows:

1. Under anaerobic conditions yeasts can ferment only certain hexoses and oligosaccharides; pentoses, for example, are not fermented.
2. A yeast that can ferment any sugar is always capable of fermenting glucose, fructose, and mannose.
3. A sucrose-fermenting yeast can also ferment raffinose, and *vice versa*.
4. A maltose-fermenting yeast does not ferment lactose, nor can a lactose-fermenting yeast ferment maltose.

But although these regularities had consistently turned up in the extensive series of tests, they did not appear to have general validity. There were a number of reports in the literature indicating a contrary behaviour of certain yeast species. None of these reports was, however, based on studies carried out with the Van Iterson-Kluyver apparatus; and in his preliminary investigations Kluyver had become acutely aware of the shortcomings – as well as of the merits – of different methods for establishing the fermentative properties of yeasts. In order to be in a sound position to evaluate the contradictory reports, Kluyver therefore secured strains of all the yeasts for which a behaviour had been claimed different from what he had observed, and these cultures were subjected to a careful scrutiny, using a variety of methods. The results will be discussed in some detail because this will provide an opportunity to call attention to certain aspects of Kluyver's scientific attitude.

The relationship between the fermentation of glucose, fructose and mannose by yeasts had been previously noticed. To Kluyver the situation seemed readily explicable in view of the closely similar stereochemical configuration of these sugars and the ease with which they

can be interconverted through their common enol form. Nevertheless, the reports indicating that some glucose-fermenting yeasts did not ferment mannose could not be dismissed offhand; and if such claims could be corroborated it would, of course, have become possible to determine mannose separately by appropriate fermentation tests.* This provided an additional incentive for a thorough re-examination of the yeasts for which a differential behaviour towards glucose and mannose had been observed. The results showed that the previous claims could not be upheld; all these strains fermented glucose as well as mannose. But the new experiments indicated why negative results had sometimes been recorded; this was usually the case when the fermentation rate was low, and the tests were conducted in an apparatus in which the inoculated sugar solution was in contact with air. Owing to the relatively high solubility of carbon dioxide in aqueous solutions, coupled with a rapid diffusion of this gas into the atmosphere, an accumulation of sufficient magnitude to cause its appearance in the form of a detectible gas phase might thus be prevented. A corollary of this situation is that the amount of yeast used as inoculum and its rate of growth, hence of fermentation, in the medium supplied may yield spurious results unless a completely closed system is used. Since the Van Iterson-Kluyver apparatus represents such a system, with mercury as the movable barrier, carbon dioxide cannot escape, and will appear as a gas phase as soon as the aqueous solution has become saturated. Under atmospheric pressure this happens when about 1 ml of carbon dioxide has been formed per ml of solution; this requires the fermentation of approximately 4 mg of sugar. If the concentration of the fermentable sugar is above 0.4 per cent, the result of a fermentation test can never be obscured by a low fermentation rate; eventually gas production will become observable. The test can be made even more sensitive by examining the result under reduced pressure.

The empirical rule concerning the fermentation of sucrose and raffinose also seemed to be challenged by the results of other investigators. But in this case Kluyver's attitude was perhaps more critical. Raffinose, as a galactose-glucose-fructose trisaccharide, possesses a sucrose configuration, which made the regularity he had observed with his own collection of yeasts readily intelligible if not downright predictable. Abandoning what thus appeared like a logical inference

* See also p. 109.

merely on the basis of a blind acceptance of the uncorroborated claims of others, even if they were eminent authorities, was never a Kluyverian attitude. Although deeply imbued with the overriding significance of experimental data, he was prone to exhibit his pronounced critical ability best when such results seemed to vitiate what appeared to be a rational hypothesis on all other counts. And, just as in the above-mentioned cases, a critical re-examination of the properties of yeast strains that had been reported to behave otherwise led to an unexceptional confirmation of Kluyver's own findings.

There was, however, no such basis for the belief that the fermentation of maltose and lactose would be incompatible. Thus the rule established could not lay claim to an expectation of general validity. But at the time the literature contained no contrary reports and it was in his own laboratory that the first examples of yeasts that can ferment both maltose and lactose were discovered many years later, when Kluyver and Custers [1940] investigated the yeasts associated with 'lambic', a special product of the art of the Belgian beer brewers. To date these *Brettanomyces* species still represent the only yeasts known to possess this property, and the genus is defined partly on this basis.

Finally the thesis contains information pertaining to two other aspects of yeast physiology, *viz.*, the fermentation of galactose, and the problem of the relation between fermentation and assimilation of various sugars.

It had long been known that yeasts, although intrinsically able to ferment galactose, sometimes do so only very slowly, or after prolonged periods of incubation. Various hypotheses had been advanced to account for this phenomenon, and Kluyver conducted some crucial experiments that supported the view that an adaptation process was involved. It was found, for example, that a yeast suspension, prepared from a culture in a galactose-containing medium, fermented galactose about equally as fast as glucose. The most intriguing experiments concerned with this problem were those in which it was demonstrated that at 40°C, a temperature at which growth is precluded but glucose fermentation is not affected, yeasts do not acquire the ability to ferment galactose at all. This established the importance of some growth process for the development of the new property. Kluyver considered it likely that this would also be reflected in the new-formation of cells, and carried out some experiments to test this. An appropriate yeast

was cultivated in a galactose-free medium, and thereafter exposed to this sugar. The appearance of the ability to ferment galactose was determined, and an attempt was made to relate this to growth as determined by cell counts. The results of these experiments were, however, inconclusive; and it was not until 1936 that the careful studies of Stephenson and Yudkin [1936] convincingly showed that the adaptation to galactose can take place in the absence of cell multiplication.

Scientifically the most interesting part of the thesis is that which deals with the studies on fermentation and assimilation of certain sugars by yeasts. It casts a clear light on Kluyver's unusual ability to perceive the difficulties that may arise out of an uncritical acceptance of experimental results that would appear to be at variance with well-established and rational ideas; to detect potential flaws in experimental procedures; to devise experiments for decisively testing the merits of alternative hypotheses; and to synthesize conflicting interpretations.

In 1910 Rose had discovered that *Endomyces magnusii*, a yeast that readily ferments glucose, fails to grow in Hayduck's medium* with this sugar as the principal carbon source, yet develops profusely if maltose, which is not fermented by this species, is substituted for the glucose. Shortly afterwards Lindner and Saito, as well as Kita, had found that a number of other yeast species behave similarly. From these results the conclusion had been drawn that glucose cannot, whereas maltose can be assimilated by such yeasts.

This situation was anomalous enough to warrant a more detailed investigation. The above-mentioned observations seemed to violate some fundamental ideas on the degradation of oligosaccharides *via* the constituent hexose units, and on the significance of fermentation as the ultimate energy source for yeast during anaerobic existence. Evidently Kluyver was reluctant to abandon these concepts, for which a considerable body of sound experimental evidence had been amassed in the course of time, and hoped that it might be possible to find a more satisfactory explanation for the aberrant results.

First of all he repeated and readily confirmed the previous experiments of Rose, Lindner and Saito, and Kita. He also found several yeasts among his own collection that exhibited essentially the same

* Tapwater, 0.025 per cent MgSO_4 , 0.5 per cent KH_2PO_4 , 0.5 per cent asparagine, 5 per cent sugar.

behaviour. Whereas *S. cerevisiae*, isolated from the baker's yeast marketed by the Netherlands' Yeast and Alcohol Manufactory, produced a crop of about 60 mg dry weight per 50 ml of Hayduck's medium with glucose, several other yeasts grew to the extent of only 1–8 mg, although they yielded crops of 20–70 mg in maltose media. But these results were not considered sufficient by Kluyver to accept Lindner's interpretation based on a differential assimilation of glucose and maltose; he looked for and finally found an explanation that did not contravene the prevailing and generally fruitful notions on sugar metabolism. The starting point was Kluyver's familiarity with the investigations of Bertrand on the often startling effects of minute amounts of certain substances on the development of diverse micro-organisms. This led Kluyver to consider the possibility that impurities in the maltose, rather than the sugar itself, might have been responsible for the good growth of yeasts that failed to develop in glucose media. The validity of this hypothesis was substantiated by experiments with carefully purified maltose; when this was used as a substrate it proved to be as ineffective as glucose for growth of the yeasts.

During the recrystallization of the maltose from 80 per cent alcohol Kluyver noticed the appearance of a flocculent precipitate; thus it became likely that it might have been this material that was responsible for the profuse growth of various yeasts in the maltose-containing media. Nitrogen determinations carried out with the original maltose, a Kahlbaum C.P. product, confirmed the presence of considerable amounts of impurities, indicating as much as 0.22 per cent 'protein'. Kluyver assumed that this would have been introduced with the amylase, presumably used in the manufacture of the maltose, and that the 'protein' was required for the growth of certain yeasts in the asparagine medium. He keenly realized that this should not be interpreted to mean that asparagine is inadequate as a general nitrogen source, and pointed out that 'it is readily conceivable that asparagine is unsuitable for the synthesis of particular cellular constituents, such as nucleic acids, for which the nitrogenous impurity can be used . . . even though asparagine may suffice for the synthesis of various other nitrogenous cell materials'.

In principle this interpretation is, of course, the one currently used to explain the need for specific growth factors by diverse organisms, and it is highly probable that the phenomenon Kluyver had here

investigated to some extent belongs in the realm of vitamin requirements by yeasts, the impure maltose being the source of such substances. That Kluyver did not further pursue the problem can easily be understood if it be remembered that the main object of the thesis was the development of a satisfactory method for quantitative sugar determinations. And, although the problem posed by the experiments of Rose, of Lindner and Saito, and of Kita had to be disposed of in order to provide a solid foundation for an interpretation of the sugar fermentation data, it was certainly not necessary for Kluyver to attempt a chemical characterization of the impurities that exerted so marked an effect on yeast growth; it was quite sufficient to show that maltose itself could not be assimilated under conditions where glucose is not.

This much may be granted. But it does not account for a curious omission in rounding out this investigation, and it is interesting to speculate on the significance of this fact. Kluyver had demonstrated that recrystallized maltose is not assimilated, and that the original preparation contained an alcohol-precipitable impurity; he had ascribed to this impurity the striking effect of maltose on yeast growth. Now it must be evident that a simple experiment could have provided direct support for this hypothesis; it would have consisted in testing the growth of yeasts that responded in the characteristic fashion to the impure maltose also in asparagine media with glucose, supplemented with the flocculent precipitate. There is, however, no indication in Kluyver's publication that this logical experiment was ever performed. Does this imply that he had overlooked so obvious and simple a check? Not necessarily; it is at least equally probable that the experiment was carried out but, contrary to expectation, had yielded negative results. On the basis of present-day knowledge of yeast nutrition such a negative result could be predicted with a high degree of probability, because it is almost certain that vitamins of the B-group were involved as growth factors for the yeasts under investigation. It is quite reasonable to suppose that these substances were present in the impure maltose. Evidence to this effect is also furnished by the fact that Kluyver failed to observe an improvement of yeast growth in Hayduck's medium with glucose to which 0.1 per cent 'Witte' peptone had been added. Now the B vitamins would not have been found in the alcohol-precipitable fraction; hence this material, too, would have

been ineffective in promoting growth. And confronted with a negative result Kluyver may well have felt that it would be advisable not to mention it in order not to introduce facts that could more easily detract from the main point than help to explain it. This would have been sound scientific strategy and prudence; and Kluyver often made use of it when experiments in his laboratory yielded results that appeared to embarrass the orderly development of a particular investigation. The only experiments in which it was shown that the various yeasts can grow abundantly at the expense of glucose pertain to yeast extract media. These had also been found satisfactory by Rose and by Lindner and Saito; but these authors had accounted for the good growth of their yeasts in glucose-yeast extract solutions by invoking a putative presence of glycogen in these media. Kluyver pointed out that this explanation fails to account for the extremely poor development of such yeasts in yeast extract without glucose, and thus concluded that, in an otherwise appropriate medium glucose can be assimilated as well as fermented.

It may here be mentioned that this early encounter with conflicting results depending on the use of pure or impure maltose preparations later aided Kluyver and Hoogerheide in dispelling an apparent anomaly in the behaviour of certain yeasts towards maltose under aerobic and anaerobic conditions, respectively. When Trautwein and Weigand had announced that *S. marxianus* and *S. exiguus*, although unable to ferment maltose, could nevertheless oxidize this sugar, Kluyver felt that this was incompatible with the views he had meanwhile developed concerning the relation between fermentative and oxidative metabolism. Hence a re-investigation of the situation was undertaken in collaboration with Hoogerheide [1933a]. From this it appeared that the rate of oxygen utilization by these yeasts in maltose solutions was dependent on the sugar concentration at levels far exceeding those at which glucose shows a similar effect; that in 10 per cent maltose solutions the rate drops sharply after relatively short periods of incubation during which the maltose concentration could not have been lowered appreciably; and that a 10 per cent maltose solution previously freed from fermentable monosaccharides by anaerobic incubation with a yeast that cannot ferment maltose no longer causes an oxygen consumption noticeably in excess of that observed in sugar-free suspensions of the two yeasts.

But it must also be remarked that it has subsequently been shown that some yeasts can actually grow at the expense of disaccharides only in the presence of air, whilst their development in the absence of oxygen is restricted to media containing a fermentable monosaccharide. In fact, Kluyver and Custers [1940], during a reinvestigation of the differential behaviour of yeasts towards disaccharides under aerobic and anaerobic conditions, established that *Brettanomyces anomalus*, *Candida parakrusei*, *T. dattila*, and *T. utilis* can grow with maltose as the sole carbohydrate only in the presence of air, and that some other yeast species exhibit a similar pattern with respect to sucrose and lactose.

THE EMERGENCE OF A PROGRAMME

Whatever importance we may attach to these studies on yeast physiology and their application, it must be realized that they constituted Kluyver's only contribution to microbiology, and practically the entire extent of his microbiological experience up to the time of his succession to Beijerinck's chair. Hence it is understandable, as already remarked in the introduction, that in his inaugural address he could only hint at some general lines of investigation, and these quite vaguely outlined, that might be pursued in his institute. During the first several months of his directorship he was occupied exclusively with preparing a background of knowledge of the field that would at least permit him to expound the fundamentals of microbiology in his courses, and to guide in an intelligent manner the work of the students who came to work under his direction. During this preparatory phase he received much support from the two assistants he had inherited from Beijerinck, L. E. den Dooren de Jong and H. J. L. Donker. In later years he was wont to acknowledge the debt of gratitude he owed them, especially the former, who had acquired a thorough training under Kluyver's famous predecessor, and possessed a considerable and integrated knowledge of the micro-organisms. Needless to say, Kluyver was an apt pupil.

The first impetus to the development of a more specific programme was provided by the fortuitous isolation of an unusual vinegar bacterium. The isolation of vinegar bacteria from beer that had become acid after exposure to the air was one of the early experiments by

which new students were initiated in the mysteries of microbiological techniques. The morphological and physiological properties of the isolated pure cultures were then further investigated, which generally permitted their identification as members of the *Acetobacter rancens* group in the sense of Beijerinck. But it so happened that in 1923 F. J. G. de Leeuw, after streaking such a beer culture on a malt extract-calcium carbonate-agar plate, obtained results strikingly different from those commonly encountered. The developing colonies caused an initial dissolution of the carbonate, due to acid production, that was far more pronounced than what was generally observed with cultures of *A. rancens*. Furthermore, the cleared areas gradually became riddled with well-developed and large crystals, in such abundance that it was relatively easy to obtain them in sufficient quantity for chemical analysis. The crystals were found to be composed of a slightly water-soluble calcium salt, readily soluble in dilute acid, from which solutions the original calcium salt could be reprecipitated by neutralization. This provided a simple and effective method for the purification of the material. The pure substance reduced Fehling's solution in the cold, and was eventually identified as the calcium salt of 5-ketogluconic acid, a product of bacterial glucose oxidation that had been discovered in 1887 by Boutroux.

A detailed study of the physiological and biochemical properties of the newly isolated bacterium [Kluyver and De Leeuw, 1924] revealed it to be a typical vinegar bacterium. It was named *A. suboxydans* to emphasize its pronounced tendency to perform only incomplete oxidations. Thus it was found to oxidize simple primary alcohols to fatty acids, secondary alcohols to ketones, poly-alcohols to their keto-derivatives, aldoses to aldonic acids, and, if the latter possessed the requisite configuration, beyond this stage to the corresponding keto-acids. Many of these properties are shared by *A. xylinum*, Bertrand's 'sorbose bacterium', but the latter, in contrast to *A. suboxydans*, usually proceeds to oxidize the products of incomplete oxidation, after these have started to accumulate, so that ultimately only carbon dioxide and water are produced.

On account of its inability to effect a further oxidation of acetic acid, ketoses, etc., *A. suboxydans* could thus be regarded as the ideal acetic acid- and 'sorbose' bacterium. It has frequently proved its usefulness for the preparation of ketoses from the respective poly-alcohols,

a procedure that was patented by Kluyver [Kluyver and Visser 't Hooft, 1931]. The efficacy of such processes is attested to by the fact that the commercial production of sorbose, an important intermediate stage in the commercial manufacture of ascorbic acid by the Reichstein method, is universally accomplished with cultures of this organism.

But the discovery of *A. suboxydans* was even more important because of the consequences it entailed for the evolution of Kluyver's biochemical outlook. First of all, it had become apparent that the physiological group of the vinegar bacteria comprises a number of representatives that are distinguishable by the extent to which they can oxidize various substrates. And the possibility of arranging these representatives in order so that the metabolic end products of one type can still serve as oxidation substrates for the subsequent ones further suggested that generally the stepwise oxidation of substrates might be analyzed with the aid of such a series of strains which would permit the isolation of the consecutive oxidation products from cultures with progressively increasing oxidative capacity. Thus came about the desire to study in a comparative manner the biochemical activities of various groups of micro-organisms.

That Kluyver anticipated a considerable clarification of our understanding of metabolic processes from such studies is clearly evident from the lecture he delivered before the Netherlands' Chemical Society in 1924. The major part of this lecture on the 'Unity and diversity in the metabolism of micro-organisms' was devoted to a discussion of the diversity; it could hardly have been otherwise because so little could be said at that time about a possible unity. Nevertheless, this aspect was also broached, and two different approaches were reviewed in some detail, *viz.*, the energetic and the chemical ones. It is unnecessary to summarize the contents of this lecture which is included in this volume; suffice it to say that it illustrates the primitive state of understanding of biochemical phenomena in general. But it may not be superfluous to emphasize Kluyver's insistence on the unifying effect that energetic considerations could exert on our interpretation of biochemical processes, equating, for example, all known cases of the so-called dissimilatory reactions, fermentative as well as oxidative, inasmuch as they represented the mechanisms whereby energy is made available for the synthetic or assimilatory, and hence energy-requiring

functions. The importance of this attitude is that it made Kluver recognize, already at this time, the fundamental problem of the manner in which the energetic coupling is brought about. The problem was posed in the form of a question; are the chemists familiar with cases in which two reactions that do not have any components in common can be energetically linked?

From time to time Kluver returned to this problem, and, as will be shown later on, he soon found himself in a position where he could make a significant contribution to its solution.

It also seems desirable to call attention to the fact that the importance he attributed in this lecture to the outcome of some experiments on the nutrition of *A. suboxydans* now seems rather misplaced. These experiments had shown that the new bacterium could grow only in media containing, besides an oxidizable substrate, complex nitrogen compounds, such as are found in peptone or yeast extract, with one notable exception: in the presence of mannitol growth was observed in an otherwise mineral medium with an inorganic ammonium salt as the nitrogen source. From these results Kluver tentatively concluded that perhaps the decrease in free energy during the first stages of the oxidation of mannitol and glucose, respectively, might be sufficiently different to enable the bacteria to utilise ammonia nitrogen for the synthesis of protein only during mannitol oxidation. We now know that *A. suboxydans* requires para-amino benzoic- and pantothenic acid for growth, so that it seems reasonable to ascribe the positive result of the culture in the mannitol-mineral medium to an inadvertent contamination with these substances. But in mineral media supplemented with the two growth factors growth also occurs if, for example, glucose or gluconic acid is supplied as the main carbon source. Hence the arguments about the interrelations between carbon and nitrogen nutrition cannot any longer be taken seriously.

In spite of this criticism these very arguments are interesting because they show that Kluver was already beginning to think in terms of what gradually evolved into the concept of 'comparative biochemistry'. The parallel drawn between the limited oxidative capacity of *A. suboxydans* with its requirement for complex nitrogenous compounds on the one hand, and diabetes as a metabolic disease on the other may have been wrong in detail; yet it embodies the principle that, in the late 'thirties, came into prominence when the nature of

growth factors and their function in specific biochemical reactions began to be understood.

Furthermore, the use of the various *Acetobacter* types may not have aided significantly in elucidating the consecutive steps involved in biological oxidations, but it cannot be denied that the approach Kluyver had suggested in 1924 is fundamentally the same as that which, more than two decades later, was employed with such spectacular success in the analysis of biochemical mechanisms, and particularly biosyntheses, with mutant strains of micro-organisms that could be graded in a sequence similar to the one Kluyver had used in arranging the different types of vinegar bacteria.

During this same period Donker [1924], in a biochemical investigation of the anaerobic metabolism of *Bacillus polymyxa*, a sporeforming bacterium frequently found in jars of improperly home-sterilized vegetables, recognized the sugar fermentation of this organism as strikingly resembling that of *Aerobacter aerogenes*, whose fermentative metabolism had been clarified by the studies of Harden, Walpole, and Norris. This outcome showed that metabolic processes of the same kind may be encountered among organisms of different morphological groups, and hence to the tantalizing notion that such instances may be enormously useful in developing new and badly needed guides for solving taxonomic problems. The possibility of considering *B. polymyxa* as the equivalent among sporeforming bacteria of the non-sporeforming *Aerobacter* species was the justification for proposing a new generic rank for the former, with the name *Aerobacillus* to indicate its metabolic relationship.

Together with the differentiation of the genus *Acetobacter* along biochemical lines, this development has undoubtedly been responsible for Kluyver's important contributions to the field of the classification of micro-organisms, and to the gradual emergence of ideas that have decisively influenced the trends in this field.

THE UNFOLDING OF THE PROGRAMME

In the course of the next two years the above-mentioned studies with the acetic acid bacteria and *Aerob. polymyxa* led to a development that signified one of the truly great advances in biochemistry. The recog-

nition of the stepwise oxidation processes, supporting the notion of differences in degree of oxidative capacity, along with the discovery of identical modes of sugar fermentation among different groups of bacteria, these were the starting points for the inception of a theory of metabolism that soon permitted a unified and gratifyingly simple interpretation of metabolic processes in general.

Following Donker's studies with *Aerob. polymyxa*, a beginning was made with a coordination of the then existing knowledge of microbial fermentations. Kluyver's outspoken desire to 'discover unity in the wild variety' soon made him perceive that all these processes can be considered as composites of a small number of major reaction types, characteristically displayed by specific groups of microbes. In the first publication on this subject eight groups of organisms were so differentiated, and these were shown to comprise only five fermentation patterns, as indicated by the products formed.

Now the possibility to arrange the acetic acid bacteria in order of ascending oxidative capacity was based on the fact that the metabolic end products of one type could be considered as intermediate products of the subsequent types because the latter can further oxidize these compounds. This raised the question whether it might not be possible to use a similar approach in attempting to systematize the extant knowledge of fermentation processes as well.

An auspicious support for such a possibility was the recognition that in diverse fermentations acetaldehyde had been postulated on good grounds as a more or less constant and crucial intermediate product; in fact, Neuberg had expressed the idea that sugar fermentations by various micro-organisms follow very similar paths. A survey of what was known and surmised with respect to different fermentations soon led Kluyver and Donker [1924a] to a synthesis that held out great promise for a unified interpretation. The extent of this synthesis can best be appreciated by considering the general pattern that emerged, and that was based on the assumption of not more than four specific reaction types, as follows:

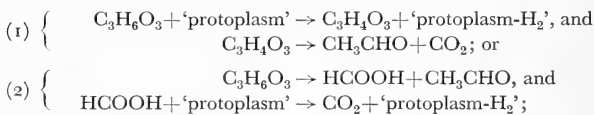
1. A primary reaction in which the hexose molecule is split into two triose moieties.
2. Transformation of the latter, either to lactic acid, or to formic acid and acetaldehyde.
3. Dehydrogenations of formic acid and acetaldehyde.

4. Condensation of acetaldehyde to acetyl methyl carbinol, or, *via* acetaldol, to butyric acid; and of acetic acid to aceto-acetic acid followed by a decarboxylation to acetone and carbon dioxide.

The dehydrogenations mentioned under 3 were depicted as proceeding under the influence of the protoplasm of the fermenting cells, yielding an oxidation product and 'protoplasm- H_2 '. Realizing that the protoplasm would have to be continuously regenerated from its reduced state, two types of suitable mechanisms were envisaged, *viz.*, *a.* regeneration through the elimination of molecular hydrogen, and *b.* by means of an interaction with a reducible compound, *i.e.* an acceptor molecule.

Seven different examples of reactions of the latter type were listed in which triose, lactic acid, acetaldehyde, acetyl methyl carbinol, butyric acid, acetone, and fructose, respectively, were reduced to the corresponding products, glycerol, propionic acid, ethanol, 2,3-butyleneglycol, butanol, iso-propanol, and mannitol, each one encountered in some fermentation process.

The possibility was considered that an additional dehydrogenation reaction, of triose to pyruvic acid, might have to be invoked. But this was not deemed necessary because the occurrence of pyruvic acid as an important intermediate product had not yet been satisfactorily established. Kluyver and Donker pointed out that the formation of acetaldehyde and carbon dioxide could equally well be represented by the reaction sequences,



the net result of these being obviously identical. A preference for one or the other would consequently have to be based on special considerations. Nevertheless, between the writing and proofreading of the paper such arguments had apparently occurred to the authors because in a footnote the remark has been inserted that a continuation of the study has made the earlier mentioned extension desirable.

Various combinations of these four simple reaction types could thus be used to interpret all known fermentation processes as the result of specific differences in the fate of the three key intermediate products,

triose, pyruvic acid, and acetaldehyde. This not only revealed the fundamental similarity and interrelations between the diverse fermentations, but it further suggested that these reaction types might also account for some peculiar phenomena, incidentally discovered during studies on fermentations under special conditions, but never before integrated with the normal fermentation patterns. Notably the 'phytochemical reductions' belonged to this category. Neuberg had coined this term to designate collectively the reduction of such utterly unrelated and 'foreign' substances as, *e.g.*, sulphur, methylene blue, or nitrobenzene, yielding hydrogen sulphide, leuco-methylene blue, or aniline, when the former were added to a sugar solution undergoing fermentation by yeast. It now seemed reasonable to interpret such reductions as the result of alternative regeneration reactions of protoplasm, the added substances competing with acetaldehyde which normally is exclusively involved in the regeneration process. This concept suggested the general possibility of deliberately steering the fate of a particular intermediate product into channels not ordinarily followed, by creating special conditions during a fermentation that would interfere with the conventional reactions. A direct experimental verification was provided by experiments that were based on the fact, shown by Neuberg and Reinfürth in 1923, that yeast produces acetyl methyl carbinol if acetaldehyde is added to a fermenting sugar solution. The carbinol is not a normal product of alcoholic fermentation, presumably because in this process the sole regenerating reaction involves the quantitative reduction of aldehyde to ethanol. But this should imply that under conditions that bring into play additional regeneration reactions, such as the 'phytochemical reductions', the aldehyde should partly escape reduction; and this fraction should therefore undergo a condensation to carbinol, which in turn might be further reduced to 2,3-butylene glycol. Appropriate experiments with yeast-sugar mixtures to which methylene blue or sulphur had been added yielded the expected results. Subsequently Kluyver, Donker, and Visser 't Hooft [1925] showed that aeration of a yeast suspension in a sugar solution also gave rise to carbinol production; this result was anticipated because it seemed likely that a regeneration of protoplasm could also be accomplished by a reaction of 'protoplasm- H_2 ' with oxygen.

Similarly those representatives of the true lactic acid bacteria in the sense of Orla-Jensen that normally produce, in addition to lactic acid,

carbon dioxide, acetic acid, ethanol, and glycerol, and had therefore been designated as 'heterofermentative' by Kluyver and Donker to distinguish them from the 'homofermentative' members that yield only lactic acid, could be induced to form acetyl methyl carbinol by cultivating them in fructose solutions. Such a behaviour had been anticipated as a consequence of the fact that the heterofermentative lactic acid bacteria were known to produce mannitol in fructose-, but not in glucose media. This mannitol formation could be considered as a particular case of a phytochemical reduction, and its occurrence would again prevent an equivalent reduction of acetaldehyde with its consequent condensation to carbinol.

Thus the sugar fermentations by different organisms had been interpreted as the net result of a small number of interrelated step reactions. The dynamic nature of these processes had been emphasized by showing that the fermentation pattern is not fixed but can be modified as a result of changes in the environmental conditions, and support for at least some of the postulated events had been provided by ingeniously contrived experiments.

But even in the first paper the analysis had already progressed well beyond this point. The impetus to a further extension had been the earlier mentioned investigation of the vinegar bacteria. Ever since 1913 Wieland had advocated the idea that the oxidation of ethanol to acetic acid by these organisms should be interpreted as a mechanism involving primarily the transfer of hydrogen atoms from the oxidation substrate, ethanol, and subsequently from its first oxidation product, acetaldehyde (in its hydrated form), to oxygen. The discovery of the various incomplete oxidations performed by *A. suboxydans* had convinced Kluyver that Wieland's theory was equally applicable to these oxidations. Hence, when it appeared that some of the central reactions in fermentations could be regarded as dehydrogenations of intermediate products by protoplasm, it logically followed that Wieland's concept could also be invoked to account for these processes, and that the subsequent regeneration reactions of the reduced to the oxidized form of protoplasm, too, were essentially similar in nature. Thus, not only the oxidations characteristic of the acetic acid bacteria, and, by extrapolation, all other biological oxidations, but even the majority of the step reactions in fermentations could be interpreted as specific

instances of hydrogen transfer mechanisms; and even the condensation reactions appeared amenable to the same interpretation.

This general theme was developed in more detail in a later publication by Kluyver and Donker in which an array of arguments was presented to show how this concept of hydrogen transfer as the fundamental feature of all metabolic processes could lead to yet further simplification and unification. Here it was also pointed out that, examined from this viewpoint, the apparently fundamental difference between fermentative and oxidative metabolic processes disappeared, and that the latter should be regarded as special cases of regeneration of protoplasm from its hydrogenated state through the transfer of the excess hydrogen to molecular oxygen. And the next step resulted in a simple and appealing synthesis of the conflicting ideas on biological oxidations that had been developed by Wieland and by Warburg, respectively. As mentioned, the former had interpreted biological oxidations as representing primarily instances of activation of substrate-hydrogen by biological catalysts, and had shown that they may proceed in the absence of oxygen provided a suitable alternative hydrogen acceptor be present. Conversely, Warburg had long emphasized the drastic inhibitory effects of substances such as cyanide, carbon monoxide, and hydrogen sulphide on biological oxidations, and defended the thesis that these effects were compatible with the inactivation of iron-containing catalysts whose mode of action was concerned with the activation of molecular oxygen. Kluyver and Donker now showed that the presumably incompatible theories of Wieland and Warburg were complementary, and, in fact, were both needed to explain the dual aspects of the mechanism of biological oxidations which should involve, at one end, an activation of substrate-hydrogen (Wieland's postulate), and, at the other, activation of molecular oxygen (Warburg's theory). Almost simultaneously, and quite independently, von Szent Györgyi in studies with plants, and Fleisch in studies with animals, had advanced the same idea. Nevertheless, it may here be stressed that Kluyver and Donker had arrived at the most general formulation in demonstrating that the Wielandian principle can be used for the interpretation not only of biological oxidations, but of any and all metabolic phenomena, whereas the Warburg principle is applicable only in those cases where oxygen is the final oxidant.

Once this universal synthesis had been achieved, the problem of

biocatalysis itself was tackled. Because the essence of every metabolic process appeared to be hydrogen activation, and because Kluver was prone to search for the ultimate in simplification and generalization, he concluded that one single property might suffice to account for the observed differences in the metabolic behaviour of diverse organisms. The most obvious feature that fulfilled such a requirement was a specific 'affinity' of the protoplasm for hydrogen.

The idea was developed as follows. A catalysed reaction involves the straining of certain bonds in the molecules of the reacting substances, a 'dislocation', as Böeseken had termed it. Now, differences in the affinity for hydrogen of the protoplasm of different organisms would cause different degrees of such dislocations, which could explain the variety of reactions that substrates and intermediate products can undergo under the influence of diverse organisms. An attractive aspect of this concept was that it eliminated the need to postulate, as was commonly done though without adequate supporting evidence, the occurrence in different cell types of a large number of haphazardly distributed enzymes. It was further pointed out that the same degree of dislocation in a molecule containing both hydrogen and oxygen atoms can result from a specific activation either of hydrogen or of oxygen, and that a definite affinity for hydrogen is equivalent to a reciprocal one for oxygen. Thus a catalyst with a high affinity for hydrogen possesses *ipso facto* a low one for oxygen, and *vice versa*. This also means that a substrate may be dislocated to a comparable extent by catalysts that possess either a high or a low affinity for hydrogen, so that the metabolic processes of characteristically aerobic organisms, representing a high affinity for hydrogen, may come to resemble those of the most anaerobic types, effecting an equivalent substrate activation through their high affinity for oxygen. Indeed, this affinity might even be so great that molecular oxygen would become firmly bound to the catalyst, thereby rendering the latter inactive for further substrate activation. This corollary provided a plausible explanation for the deleterious effect of oxygen on obligatory anaerobes.

It is superfluous to enter here into a detailed discussion of the application of the principles of hydrogen activation and transfer to a large number of particular metabolic processes; this can be found in the classical paper by Kluver and Donker, 'Die Einheit in der Biochemie', that has been reprinted in this volume.

FURTHER DEVELOPMENTS: PHOSPHORYLATION AND
THE NATURE OF ASSIMILATION

The successes scored in interpreting the vast diversity of metabolic processes in a simple and unified manner understandably induced a desire more rigorously to test the applicability of the principles to specific cases. Among the first to be so investigated was the mechanism of alcoholic fermentation. In part this may have been owing to Kluyver's earlier preoccupation with this fermentation; but in part it must also be ascribed to the recognition that an as yet mysterious and unexplained phenomenon had been observed in this fermentation. The concept of hydrogen transfer had already made it possible to integrate the carbinol condensation and the phytochemical reductions with the more normal reactions. And the one aspect that had so far remained outside the scope of speculations now promised to shed some further light on the introductory conversion of a fermentable hexose molecule into the postulated two triose molecules.

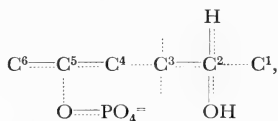
For nearly twenty years it had been known that the fermentation of sugar by yeast press juice or maceration juice is accompanied by the intermediate formation of hexose phosphate esters, and on the basis of quantitative experiments Harden and Young had established a relationship between the formation of carbon dioxide and alcohol on the one hand, and of hexose phosphate on the other, that could be expressed by the equation:



However familiar we may be with the current interpretation and significance of this equation, it should be remembered that in the 'twenties it did no more than paraphrase an over-all result, and that, taken at face value, it might suggest that concomitantly with the esterification of one sugar molecule another one was broken down to carbon dioxide and alcohol; a rational interpretation had not been suggested.

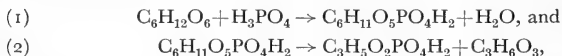
It is equally significant to realize that during the early 'twenties great strides had been made in our understanding of the structure of sugars; Haworth, Irvine, and their collaborators had established the ring structure of hexoses. It was this aspect that caused Kluyver to look for a potential connexion between the act of phosphorylation and the subsequent rupture of a hexose molecule into two triose moieties

which might furnish an intelligible interpretation of the Harden and Young equation. Following the same line of reasoning that had been so successful in making metabolic processes more easily comprehensible as a result of the dislocation of substrate molecules under the influence of catalysts with appropriate affinities for hydrogen, it soon occurred to him that the ring configuration of a sugar, representing by far the most stable one, might prevent an effective attack by a biological catalyst, and that a primary modification of the structural pattern might be a prerequisite for initiating a subsequent break of the molecule between the third and fourth carbon atoms. In coupling this notion with the equally plausible one that Harden and Young's ester, as a hexose diphosphate, would probably be formed as a result of two consecutive processes, involving hexose monophosphate as a first product, the concept emerged that the formation of this monophosphate *per se* might be the means by which the structural modification was accomplished. Due to the strongly polar nature of the phosphate group the first phosphorylation would cause a redistribution of various bond strengths, and by assuming that this esterification involved the hydroxyl group at carbon atom five after opening of the ring, a case could be made out for the specific weakening of the central carbon-carbon bond, as indicated in the following diagram:



where the dotted lines represent weakened, the dash-dotted ones reinforced bonds. As a consequence of the specific activation of hydrogen attached to carbon atom four, this hydrogen could then be transferred to carbon atom three, at the same time causing a rupture of the carbon chain.

This ingenious hypothesis implied, therefore, a reaction sequence of the kind,



in which a triose molecule is generated alongside a triose-phosphate molecule as a direct consequence of the primary esterification. Under

the dislocating influence of the protoplasm the triose would be rapidly converted into carbon dioxide and alcohol; the triose phosphate would first have to be de-esterified before it, too, could yield the final fermentation products.

By further postulating that the rate of hydrolysis of the triose phosphate by yeast juice is smaller than the rate of formation of hexose monophosphate, of its decomposition to triose and triose phosphate, and of the triose breakdown, a result was obtained that was in full agreement with experimental observations. In the first place it explained why the sugar decomposition was inextricably linked with the formation of phosphate esters; the latter served in effect to prepare the sugar molecule for its eventual breakdown. Secondly, the rapid formation of triose phosphate and triose from the hexose monophosphate, and the equally rapid disappearance of the triose, served to account for the fact that, as Harden and Young had established, per molecule of esterified phosphate one molecule of carbon dioxide and ethanol each were generated. Thirdly, the low rate of hydrolysis of the triose phosphate would cause this product to accumulate, so that gradually an increasing amount of inorganic phosphate would become tied up; following its complete disappearance the rate of production of carbon dioxide and ethanol must needs become dependent on phosphate liberation and the simultaneous triose formation by the dephosphorylation reaction. Fourthly, the accumulation of triose phosphate, according to the proposed mechanism of its formation possessing the structure of 2-phosphoglyceraldehyde, would result in its condensation to hexose diphosphate in a manner completely analogous to that of the condensation of acetaldehyde to acetyl methyl carbinol which is observed under conditions of aldehyde accumulation. And finally, the hexose diphosphate so formed should be a keto-derivative, and the identical one regardless of whether the fermentable sugar were glucose, fructose, or mannose. That this was so had been established by Harden and co-workers.

Without performing a single experiment, the available information had thus been used to develop an hypothesis that, for the first time, had accomplished an interpretation in which the formation of phosphate esters had been incorporated as a logical and indispensable element in the picture of alcoholic fermentation. It stands to reason that the same mechanism was proposed to explain the initial stages of sugar

degradation by any and all organisms that did not obviously effect a direct oxidation of hexoses to hexonic acids.

Soon after the publication of the paper by Kluyver and Struyk [1926] in which this interpretation was advanced, it was adopted by all the leading investigators of carbohydrate biochemistry. But six years later the hypothesis, then no longer in keeping with newly established facts, was abandoned, and hexose diphosphate once again assumed the place of prominence it had lost. After another six years Lipmann had begun to perceive the significance of phosphorylation as a means of preserving metabolic energy in the form of 'high energy phosphate bonds'.

And Meyerhof [1945, 1947] provided a new and more refined explanation of the Harden and Young equation, based upon the lack of a sufficiently high ATP-ase activity of the yeast juice, a result of the fact that in the crude extracts this enzyme is quite labile and that it is strongly adsorbed on the structural elements of the yeast cells, so that only a small proportion of the total ATP-ase content of intact yeast appears in the extracted juice.

Meanwhile the applicability of the principles developed in the course of 1924-1926 was tested for many different types of fermentations, and it cannot be denied that they rendered excellent service in helping to account for the butyric acid and butanol-acetone fermentations, the fermentations of the coli-aerobacter group of bacteria, the propionic acid fermentation, and the anaerobic processes of denitrification and sulphate reduction.

In fact, these ideas were soon recognized as being of quite fundamental significance. In consequence Kluyver was invited to deliver a series of lectures before the University of London, and two years later at Iowa State College in the U.S.A. The London lectures, given in 1930, were published in book form by the University of London Press in 1931. They contained a simple and highly condensed account of the principles underlying the 'unity in biochemistry'. It is here that for the first time the term 'comparative biochemistry' was launched, with the remark that, though as yet little developed, 'this line of study . . . may in future win the same significance for biochemistry as "comparative anatomy" has already long ago attained for anatomy'.

Here, too, one can find the four equations that represent the various

possible types of hydrogen transfer reactions in their most generalized form, as:

- (1) $AH + B \rightarrow A + BH;$
- (2) $AH \cdot B \rightarrow A \cdot BH;$
- (3) $AH \cdot B \rightarrow A + BH;$ and
- (4) $AH + B \rightarrow A \cdot BH$

And here, finally, Kluver developed his matured version of the concept that there is no need to assume a fundamental difference between those aspects of metabolism that had been so carefully differentiated in the 1924 lecture, *viz.*, catabolism and anabolism.

To be sure, the nature of assimilatory processes had already been discussed by Kluver and Donker in 1926, in Part VI of 'Die Einheit in der Biochemie', where it had been stated that the principle of hydrogen transfer can adequately account also for the synthesis of the major cell constituents, the carbohydrates, fats, and proteins, from the substrate molecules. The same theme was treated more fully in the paper Kluver [1930] contributed to the first volume of the newly established 'Archiv für Mikrobiologie', on the interrelations between fermentation, oxidation, and assimilation. Now no longer pre-occupied with the problem of how to conceive of an energetic coupling of assimilatory and dissimilatory processes that did not have one or more components in common, Kluver here reasoned that dissimilation should be considered as the means whereby, through oxidation-reduction reactions, molecular entities are produced that have a higher energetic potential than the substrate itself. The formation of acetaldehyde during the degradation of sugar was used as an example; through aldol condensations, dehydrations, and reductions, this substance could be converted into fatty acids in a manner fully compatible with mechanisms encountered in the spontaneously occurring catabolic reactions. Since the formation of glycerol from sugar, as, for example, in yeast fermentation, is also part of a typical catabolic process, the synthesis of fat from sugar could be understood as the result of a sequence of step reactions none of which involved principles different from those used in explaining the spontaneous, energy liberating catabolic conversions. It had therefore become superfluous, and even appeared ill-advised, to postulate that biosyntheses cannot proceed without a special influx of energy; rather should one interpret the

relation between breakdown and synthesis by considering the former as the means whereby are supplied the energetically elevated building blocks from which the synthetic reactions can proceed spontaneously.

If we compare this concept with present-day knowledge it will be clear that there has not been a fundamental change in outlook. To be sure, our understanding of the intimate details of biochemical mechanisms has been immensely increased and broadened. We no longer think in terms of acetaldehyde as a key intermediate product, for example. Nonetheless, the mere substitution of 'acetyl-coenzyme A' for this substance is all that is required to show that the latest ideas on the mechanism of fatty acid synthesis are virtually identical with those that Kluver had suggested more than 25 years ago. If we furthermore remember that acetyl-coenzyme A formation is currently interpreted as the direct result of catabolic conversions, it is evident that we have achieved a much more detailed, but not a fundamentally different comprehension of the mechanism of synthetic processes.

This concept of assimilation and dissimilation as inextricably linked aspects of what Kluver called 'metabolism one and indivisible' could have been experimentally tested, for example by determining the amount of growth (assimilation) of particular organisms in media with various substrates, inclusive of those which, as intermediate products, were presumed to be used as the direct participants in the synthetic processes. But it was generally believed that the amount of cell material formed in simple media is very small compared to the quantity of substrate used, which made it unlikely that accurate experiments of this sort would be feasible. Another deterrent to such studies was the high volatility and toxicity of acetaldehyde, one of the most crucial amongst the intermediate products.

This situation was changed when Barker [1936], in studies on the oxidation of small amounts of substrates by resting cell suspensions of *Prototheca zopfii*, found that the quantities of oxygen consumed and carbon dioxide produced were far short of those required for complete oxidation of the substrates, and interpreted these results, as well as earlier ones obtained by Cook and Stephenson [1928] in similar studies with *B. coli*, to mean that the oxidation was accompanied by an assimilation of considerable magnitude, sometimes amounting to more than half of the substrate carbon. The significance of this phenomenon did not escape Kluver, and soon thereafter his collaborator, Giesber-

ger [1936Th], conducted numerous comparable experiments with various *Spirillum* species. The report of this investigation contains an interesting discussion in which the notion of a 'chip respiration' was launched. This term was introduced to convey the idea that the oxidative dissimilation of a substrate might represent mainly a whittling down of its molecules to fragments that could be used as appropriate building blocks for the synthesis of cell materials. The parts that were intrinsically unsuited for this purpose would thus be eliminated as 'chips', generally in the form of carbon dioxide. The experimental results were, however, not entirely consistent with this idea; some of the over-all equations, expressing the quantitative relations between the amounts of substrate used, oxygen consumed, carbon dioxide evolved, and 'assimilation products' formed, were difficult to interpret in this manner. The only conclusions that could safely be drawn from the data were that the oxidative degradation of an organic substrate is quite generally accompanied by a massive assimilatory activity, and that, even in the absence of assimilable nitrogen compounds, when protein synthesis and hence cell multiplication is precluded, a variety of assimilation products may be formed.

Shortly afterwards Clifton [1937], in Kluyver's laboratory, extended these studies, and discovered that this assimilatory activity can be prevented if the resting cell suspensions are exposed to low concentrations of dinitrophenol or sodium azide, which do not appreciably inhibit the oxidation of the substrates. Evidently the connexion between degradation and synthesis is severed under the influence of these compounds. Not until more than a decade later Loomis and Lipmann [1948] found an explanation for this effect by showing that dinitrophenol blocks the formation of adenosine triphosphate that normally results from the transfer of phosphate from phosphorylated intermediate products such as acetyl phosphate. In current terminology, and entirely in keeping with Kluyver's concept of the nature of biosynthetic processes, this can be paraphrased by saying that under these conditions the necessary raw materials for anabolic reactions are prevented from being formed during the oxidation of the substrates.

THE NATURE OF BIOCATALYSIS

The developments mentioned in the last few pages show how firmly our current notions of metabolic reactions are based on the general concepts that Kluyver had advanced.

Whenever a particular branch of science has reached the stage where such a generalization has been achieved, further progress is apt to result primarily from attempts to comprehend in an increasingly refined manner the mechanism underlying those phenomena that have been recognized as the most basic ones. Ultimately this approach should permit an interpretation of the behaviour of inanimate as well as animate systems in terms of the properties of the elementary particles of matter, or of whatever may eventually take their place as the most fundamental constituents of the universe.

Thus, when Kluyver had enunciated the main principles of biochemistry, it was logical to anticipate that important new advances in this field would come from studies aimed at acquiring a more profound understanding of the nature and mechanism of biocatalysis. Amongst the possible approaches that could be envisaged in 1930, one was obviously directed at investigations of the biocatalysts themselves. How much has been accomplished in this respect, owing to twenty-five years of increasingly intensive enzymological research, need not be discussed here. But Kluyver was reluctant to follow this line, with the consequence that not until the last few years were enzyme-chemical studies conducted in his institute. And it is pertinent to our subject to examine into the reasons for this attitude.

At the time when the principles of biochemistry were gradually being developed, knowledge of enzyme chemistry was virtually nonexistent. To be sure, the hydrolysis of starch under the influence of saliva had been known for about a century, and subsequently the hydrolysis of other complex carbohydrates, of fats, and of proteins by tissue extracts had been established. The agencies responsible for these hydrolyses appeared to be catalysts produced and often excreted by living cells; even if not excreted they could usually be extracted with ease. They had been designated as 'ferments' or 'enzymes'; they seemed to exhibit a high degree of specificity; but their chemical nature and mode of action were completely unknown as late as 1920. The inactivation of enzymatic activity by exposure to tem-

peratures that caused protein denaturation had suggested that enzymes might be proteins or substances associated with proteins, and during the early 'twenties Willstätter and collaborators had assembled a mass of information in support of this view. Finally, in 1926, Sumner had announced the isolation of a crystalline protein that possessed the power to hydrolyze urea to ammonium carbonate, thereby establishing that urease, at least, was a typical protein.

It is understandable that the existence of hydrolytic enzymes had led to the assumption that comparable biological catalysts would be involved in other types of reactions brought about by living organisms. And when Buchner had prepared, from ground-up yeast, a cell-free press juice that could still promote a characteristic alcoholic fermentation of sugar, this was hailed as proof positive for the enzyme theory of fermentation. Some early successes were booked in preparing from other organisms cell-free juices that caused the formation of lactic acid from sugar, or an oxidation of alcohol or glucose. But the extraction of such enzymes invariably proved more difficult than that of the hydrolases; it required the use of large quantities of cells, and, because these enzymes were not excreted, they had to be liberated by rupturing the cells. In consequence the composition of these enzyme extracts was exceedingly complex, approximating that of the cells themselves. Now this marked difference in the behaviour of enzymes involved in hydrolyses and in typical dissimilatory reactions, respectively, had already been commented on by Kluver in his 1924 lecture on the 'Unity and Diversity in the Metabolism of Micro-organisms'. Here he had stressed what appeared to him the significant fact that hydrolyses represent preparatory events whereby large, non-diffusible molecules are broken down to small and readily diffusible units, and that, from the point of view of cellular economy, they are devoid of energetic significance. In contrast, the typical dissimilation processes are primarily concerned with energy provision of the cells, which accounted for the fact that the causative enzymes had to be rigorously cell-bound.

Kluver was not averse to considering dissimilatory transformations as enzymatic; in fact, in a series of lectures delivered in Amsterdam in 1929 he had categorically stated: 'Biochemistry in its entirety is enzymatic'. Why, then, did he show so little inclination to include enzymological studies in his programme? There were two different factors to which we can assign an important role.

The first was his general philosophical outlook which made him wary of the tendency to explain biochemical reactions in terms of events mediated by specific enzymes. As long as there was no prospect that the chemical nature of the postulated enzymes could be elucidated or even approximated, this practice seemed like futile paraphrasing without adding anything to our understanding of the processes themselves. Worse, this approach, which had already induced many biochemists to name a specific enzyme for every biochemical reaction that could be surmised to exist, might easily lead to a false sense of comprehension, and to a mental image of a living cell as the depository of a vast number of such catalysts, distributed without rhyme or reason; a bag of enzymes rather than a smoothly functioning unit. Alternatively, the development of general principles that could help to account for the multifarious activities of living organisms in a chemically intelligible manner had given rise to the concepts of hydrogen transfer and the unity in biochemistry, and eventually to the notion that a single property should suffice to explain the many conversions a cell is capable of performing. This approach was clearly antagonistic to the one based upon the assumption of a haphazard multiplicity. It had, moreover, been fruitful; it was dedicated to a search for still greater unification; and it could be further exploited. If finally we remember the difficulties inherent in procuring extracts of nonhydrolytic enzymes, we can readily appreciate Kluyver's belief that enzyme research would not be particularly rewarding.

Secondly, this attitude appeared to be vindicated by the results of the investigation on the coenzyme of alcoholic fermentation that he carried out with the collaboration of Struyk [1928]. As already stated, the exceedingly complex nature of an extract such as zymase made it seem hopeless to attempt a chemical characterization of the specific catalyst. But Harden had discovered that a yeast juice could be deprived of its fermenting capacity by dialysis or ultrafiltration, and that the residue could be restored to activity by the addition of the dialysate or filtrate. Thus the factor in the dialysate responsible for this behaviour was evidently a substance of relatively small molecular size; in addition, it had been found resistant to prolonged boiling. These properties of the so called co-zymase indicated that it would be more amenable than zymase itself to chemical investigation, and some bio-

chemists, notably Meyerhof and von Euler, were already engaged in studying the nature of this material.

Now, the theory of alcoholic fermentation that Kluyver and Struyk had developed called for the participation of two different kinds of catalysts, *viz.*, a phosphorylating and an hydrogen activating agent. Besides these nothing else should be needed with the possible exception of a substance that could serve as the initial hydrogen acceptor for the conversion of methyl glyoxal into pyruvic acid. According to the theory, acetaldehyde should be suitable for this purpose, and Meyerhof, in fact, had already shown that a dialysed yeast juice could sometimes be reactivated by the addition of trace amounts of this substance. Meyerhof had also found that the fermentation induced by a zymase preparation was frequently accelerated by small amounts of hexose diphosphate. This, too, could be explained within the framework of Kluyver and Struyk's theory by assuming that the phosphorylation would proceed more effectively with the hexose ester than with inorganic phosphate.

So far the experimental results did not present serious obstacles to a simple explanation of the co-zymase effect. But it had also been established that zymase preparations, after ultrafiltration and prolonged washing, yielded an inactive residue that could no longer be reactivated by hexose phosphate and acetaldehyde, although boiled yeast extract or the concentrated dialysate did restore the activity. Thus the problem was raised how this presumably more genuine co-zymase effect could be explained. Kluyver believed that the answer would be found in earlier studies of Buchner, Hahn, and others, who had postulated that the decline in fermenting capacity of a yeast juice was due to a decomposition of the zymase itself by proteolytic enzymes present in the original juice. This hypothesis could easily be elaborated to explain the reactivation of a zymase residue; it was but necessary to assume that the boiled yeast extract could in some manner protect the zymase against proteolytic degeneration.

Numerous preliminary experiments seemed to support this explanation. Nevertheless, the reactivation of a thoroughly washed ultrafiltration residue was always accomplished by the addition of materials that could not be guaranteed to have been entirely devoid of co-zymase. And a crucial experiment that could serve to abolish the notion that an additional and functionally mysterious factor was need-

ed should carry precisely such a guarantee. This consideration led to the successful experiments in which it was shown that a filtered zymase residue, no longer capable of fermenting sugar, even when supplemented with hexose diphosphate and acetaldehyde, and hence co-enzyme free by the most rigorous standards, could be reactivated by mixing it with a boiled aliquot of the same preparation. This result was interpreted to mean that the boiled extract, in which the proteases had been destroyed, contained protease-inhibiting materials. The alternative explanation that a low-molecular weight substance needed for fermentation, a true coenzyme as we now understand this term, could be generated by boiling co-enzyme-free zymase, was not considered; in view of the fact that nothing was yet known about the nature and function of coenzymes this is not surprising.

The conclusion of this extensive investigation was, therefore, that the co-enzyme effect could be attributed to one or more of the following factors:

1. an initial hydrogen acceptor function, such as exhibited by acetaldehyde;
2. an activation of the phosphorylating principle by hexose diphosphate; and
3. protection of zymase against proteolytic degeneration.

Thus it appeared that the term co-enzyme had actually been applied to three entirely different types of substances, with different modes of action. For this reason Kluver and Struyk argued that it would be best to 'banish the notion of a coenzyme altogether from the literature, and to replace it by the much more rational concept that a cell-free fermentation requires, in addition to zymase and phosphatase, the presence of an initial acceptor, of an hexose phosphate ester, and of substances that can regulate the proteolysis of the zymase system. . . . Although the function of the coenzyme had thus far remained entirely obscure, it is now possible to indicate the nature of its activity, and even to link it with phenomena whose importance for the cell-free fermentation had long been recognized. . . . Further cogitations then lead to the idea that the indispensability of the proteolysis-regulating factors should be ascribed to the fact that, for its maintenance and for the normal execution of its functions, the living cell requires the harmonious cooperation of the principles it contains. If this harmony is disrupted, as happens during the isolation of the zymase, and

even more during the subsequent filtration and washing, the living substance undergoes selfdestruction.' [Kluyver and Struyk, 1928. p. 258].

It is not difficult to understand that this investigation decided Kluyver against undertaking any further studies in the field of coenzymes. And, despite the great advances in our knowledge of biochemical reaction mechanisms resulting from the eventual isolation and chemical identification of various coenzymes – advances that Kluyver fully appreciated and readily acknowledged – he never quite lost his earlier dislike for such studies. Even as late as 1954, in the second of the Harvard lectures, he conceded this in the passage: '... it is tempting to say a few words about the significance of the discovery of a coenzyme being involved in a certain reaction step. I have to confess that my first mental reaction on hearing of such a discovery is to lower the flag to half-mast' (p. 47).

An additional effect of this study on 'the so-called coenzyme of alcoholic fermentation' was to strengthen still more his belief that studies with enzyme extracts were not apt to contribute much to biochemical comprehension. Even if – and this was doubtful enough in the 'twenties – better techniques could be invented for the preparation of cell-free extracts, it seemed to him improbable that studies with such extracts would contribute anything beyond what had already been inferred from investigations with intact organisms. The studies on the cozymase problem afforded an instructive example; it was the previously developed theory of alcoholic fermentation that had led to predict what the coenzyme should be and do, and this had been supported by experiment. Meanwhile, these same studies had failed to provide a deeper insight into the mechanism of the process, and rather emphasized the disadvantages inherent in working with extracts in which the harmonious cooperation of various principles had been deranged. On the other hand, the concept that the essence of biocatalysis was the activation and transfer of hydrogen suggested that a more fundamental approach could be devised which would lead to a sounder organization and understanding of the various manifestations of the chemical activities of living organisms. This was developed in the last of the earlier mentioned London lectures.

Here, Kluyver first argued that there is no ground for believing that some biochemical events might not be mediated by enzymes,

concluding that 'it has become very difficult to deny the enzymatic nature of those dissimilation processes for which the experimental proof is still lacking. For instance, I doubt if anyone would seriously maintain that sugar breakdown by the yeast cell is an enzymatic process, whereas the analogous fermentation by *B. coli* has a non-enzymatic character' (p. 94). And what was true for dissimilatory reactions should hold equally for assimilation processes; after all, the synthesis of a new carbon-to-carbon link is encountered also in typical catabolic reactions. Nevertheless, he perceived that this thesis might be less readily accepted, and proceeded to disarm his potential opponents: 'But you will perhaps observe that whilst it is easy to ferment sugar with the aid of the enzymes of yeast, it has not as yet been found possible to synthesize fat from sugar with these enzymes, a conversion which is nevertheless of general occurrence in the living yeast cell. However, on second thoughts this is not to be wondered at. For I have only to remind you how the conversion of sugar into fat demands for its successful completion a harmonious succession of a special set of primary reactions out of the many that are possible. And the perfect harmony which is the one condition for such a long chain of reactions is the exclusive prerogative of the living cell' (p. 95).

Next he considered the implications of the enzymatic interpretation of metabolism:

'The question then arises whether we have to conclude from the foregoing that a living cell should be considered as an arsenal filled up with enzymes, which successively are brought into action. Such a supposition would only be justified if every chemical reaction brought about by the cell required its own specific catalyst.

'A survey of modern enzymological literature shows that the doctrine of the extreme specificity of enzymes is adopted by almost all the leading authorities in this field. . . . All the same, it seems worth while to dwell for a moment on the problem whether this doctrine is actually well founded. . . . We should then like to observe in the first place that a catalyst can only be expected to be non-specific, *i.e.* be able to promote various reactions, in so far as these reactions are of the same nature. Now, we have seen that the primary reactions to which biochemistry can be reduced satisfy this demand in a high degree; all these reactions are either of the hydrolytic or of the oxidoreduction type. So at first sight it seems quite conceiv-

able that the same enzyme can promote several different conversions' (p. 95-96).

He then pointed out that catalysis involves the formation of a loose compound between catalyst and substrate, resulting in an activation of the latter. Thus specificity might be conditioned either by configurational patterns or by the degree of activation which depends on the electrostatic properties of the catalyst. Hydrolases would tend to exhibit the former kind of specificity, whereas the oxido-reductases, acting on relatively small molecules and causing reactions with pronounced energetic effects, should display specificity largely because of differences in the intensity of their electric fields. Kluver concluded with the statement: 'Ignoring all this, the experimental material gathered with hydrolases has led biochemists to extend unconsciously the doctrine of great specificity to the whole field of enzymes . . . For every biochemical oxido-reduction . . . the existence of a special "ase" has been postulated. In this way biochemical youth is nowadays poisoned by the necessity for learning numerous names of the barbaric succinofumarase and quercito-pyrogallase type.

'However, such a procedure is only practicable as long as the number of substrates which are liable to dehydrogenation under the influence of one and the same specific cell is limited. But when we think of the bewildering diversity of compounds which are able to act as dehydrogenation substrates for the cells of *Pseudomonas putida*, . . . it will be generally agreed that here the doctrine of extreme specificity becomes untenable. For it can scarcely be conceived that the cells of the bacterium in question contain as many dehydrases* as there are suitable oxidation substrates for these cells. And, moreover, we should be obliged to assume that these cells have at their disposal specific catalysts for substrates such as bromo-succinic acid and bromopropionic acid which do not occur in nature, and which are only made by the conscious operations of the organic chemist.

'So in this case there is not the slightest doubt that one and the same catalyst is capable of acting upon different substrates and very probably even on a large number of these. Once accepting the presence of such dehydrogenating "master keys" in bacterial

* *Ed. note:* meant is obviously dehydrogenases.

cells, there is no clear reason why one should not go farther and accept the supposition that in *Pseudomonas putida* there is only one single oxido-reduction promoting agent which acts on all the substrates . . . And the same conclusion holds good for all the primary oxido-reductions which together constitute the typical fermentation process of a cell.

'This does not imply, however, that no specificity at all exists in oxido-reduction promoting agents. On the contrary, we shall have to seek, in the differences of the electrostatic properties of the agents of different specific cells, the explanation why some of these cells dehydrogenate sugars only, others hydrocarbons as well, still others methylamine or nitrites. And we may cherish the hope that the time will come when a wellfounded quantitative theory of catalysis will lead to a sharp characterisation of the electrostatic properties of the different catalysts, and in doing so make it possible to predict the nutritional requirements of the corresponding cells' (p. 98-99).

There is much in this reasoning that is as appealing as it is sound. Ironically, the argument about a single dehydrogenating agent for a large number of substrates has gained the strongest possible support from the isolation of coenzymes I and II, their identification as di- and triphosphopyridine nucleotides, and the finding that these are the molecular species directly involved in the dehydrogenation of numerous substrates. Admittedly, enzymological studies have also established that these coenzymes function only in cooperation with specific protein apoenzymes, so that the assumption of a multiplicity of enzymes is still a necessary adjunct hypothesis. But this aspect has gradually lost some of its perplexing consequences; the discoveries pertaining to the apparently universal occurrence of common pathways, of a small number of cyclic mechanisms, and of the phenomenon of induced enzyme synthesis have eliminated the need for assuming that a particular cell is invariably equipped with as many types of specific protein molecules as there are substrates it can utilize.

In reading Kluyver's papers of this period during which he formulated the general principles of metabolism, one may perhaps find the approach too theoretical and speculative. This was also the criticism of some of his contemporaries, one of whom complained that what was really needed was 'more matter and less art'. Kluyver was fully aware of this; but his philosophical inclination always made

him search for concepts that could help to tie various known facts together, and he was wont to defend this attitude by quoting the felicitous phrase of A. V. Hill, 'It is dangerous to speculate too far, but it is foolish not to speculate at all'.

It cannot be denied that, if an hypothesis can be made to accommodate apparently unrelated or even conflicting observations, the tendency to speculate is sometimes conducive to generating a feeling of confidence rather than to performing experiments, especially such as might throw a different light on a particular problem. A case in point is the earlier discussed investigation on co-zymase. Surely the mechanism of alcoholic fermentation proposed by Kluyver and Struyk, however much of an advance it represented over other theories current at the time, still left a great deal to be explained. And the general state of ignorance concerning the composition and mode of action of enzymes was no justification for the tacit assumption that there should be no need for the cooperation of small molecules with as yet unexplained functions.

Be that as it may, it is impossible to determine at just what point speculation will become dangerous. Moreover, the danger can easily be exaggerated, and in any case it is less so to science than to the speculator, who may be entranced by a preconceived notion to the point of not recognizing alternative possibilities. But since scientists, like microbes, vary a great deal in their predilections, there will always be some who are not so taken in, and, by following other approaches, provide the information that is essential in checking and correcting assumptions that lack a sufficient experimental basis. For science is a curious amalgam of art and matter, and its most outstanding characteristic is that rival hypotheses are ultimately resolved not by personal preference but by the force of impersonal facts; accumulated observational evidence is the only final arbiter. This does not mean, however, that the elements of art and imagination can be dismissed as irrelevant in scientific pursuits; they are as essential as factual data because it is through their exercise that extant knowledge can be integrated and new data can be collected in a meaningful manner. Besides, the search for general relationships has the refreshing effect that it leads to interpreting isolated events as coordinated phenomena, and thus guards against getting bogged down in details.

Kluyver, who was a master in the art of 'discovering unity in the wild variety of nature', had meanwhile started the search for a 'quantitative theory of catalysis' that 'could lead to a sharp characterization of the electrostatic properties of different catalysts', and 'might make it possible to predict the nutritional requirements of the corresponding cells'.*

It was hoped that a study of the redox potentials established in metabolizing cultures of various micro-organisms would provide a promising approach to this problem, the underlying idea being that the specific affinity of the cells for hydrogen would express itself more or less clearly in the quantitatively measurable potentials.

In a publication on redox potentials from his laboratory [Elema, Kluyver, and Van Dalfsen, 1934] the nature of the general problem was introduced as follows:

'... a closer study of the connexion between the redox potentials established in microbial cultures and the nature of the metabolic processes had long been part of the programme of our institute. This was a logical consequence of the attempt to reduce the totality of biochemical events to chains of catalytic oxido-reduction reactions.

'In choosing specific processes for studies of this sort we were guided by the following considerations. Firstly, it was necessary to select a metabolic process that can occur under fully physiological conditions in a medium of very simple composition. Secondly, it seemed essential for the time being to neglect the aerobic metabolic processes because under the usual conditions of cultivation these lead to heterogeneous situations in the culture medium. The slow diffusion of oxygen into the deeper layers of a culture liquid, and the consequent uneven distribution of the cells in the cultures will surely result in differences in redox potentials at different distances from the surface.

* The meaning of this latter phrase may easily be misunderstood by the younger microbiologists and biochemists. In 1930, when our knowledge of specific growth factors was as primitive as that of enzymes, Kluyver could not have used the term 'nutritional requirements' in its current sense. From the context, and from knowledge of the evolution of his ideas, it is obvious that he had in mind the possibility of predicting which of the great variety of potentially oxidizable or fermentable substrates – inorganic substances, hydrocarbons, alcohols, polyalcohols, fatty and substituted acids, carbohydrates, aromatic compounds, amines, amino acids, etc. – a particular organism should be able to utilize.

'Amongst anaerobic metabolic processes it seemed that particularly denitrification should fulfill the above-mentioned requirements' (p. 319).

A beginning was therefore made with a study of the redox potentials in cultures of denitrifying bacteria. The results clearly indicated that the measured potentials were indeed dependent on the nature of the metabolic process. During the first phase of the cultures, when only nitrate was present, a potential of around -100 mV was established; when nitrite had accumulated the potential rose to a level of -40 mV; thereafter it gradually dropped again, eventually and quite sharply to a new low level of about -260 mV, coinciding with the disappearance of the nitrite. At this stage the addition of nitrate or nitrite sufficed to restore the earlier established potentials.

On account of the drastic changes in hydrogen ion concentration during the development of cultures of denitrifying bacteria, and their inevitable effect on the redox potential, the results obtained were not interpretable in terms of absolute values; nevertheless, they showed that the type of metabolic process was reflected in the measured potentials.

In passing it may here be mentioned that these studies had also led Elema in Kluyver's laboratory to discover the reversible two-step oxidation of reduced pyocyanine and chlororaphine, simultaneously and independently found by Michaelis in the U.S.A.

Spurred on by these promising results, the programme was soon expanded by Kluyver and Hoogerheide [1934] to studies on the potentials established in suspensions of micro-organisms provoking a characteristic alcoholic fermentation. The organisms used included various yeast species as well as the bacterium, *Pseudomonas lindneri*, which Lindner had discovered as the causative agent of the alcoholic fermentation of agave juice from which the Mexicans prepare the alcoholic beverage known as 'pulque'. This bacterium had been thoroughly studied by Kluyver and Hoppenbrouwers [1931] who had shown that it can ferment sugar to equimolar amounts of carbon dioxide and alcohol, accompanied by small quantities of lactic acid, and that, in contradistinction to yeasts, it can ferment glucose and fructose, but not mannose, so that it can be used for the quantitative determination of mannose in sugar mixtures by means of the previously discussed fermentation method.

Under anaerobic conditions all of these organisms, in suspensions containing a fermentable sugar, caused the establishment of a redox potential of 80–100 mV. The concordant data therefore suggested that this is the potential characteristic for alcoholic fermentation. Together with the marked difference in the potentials of cultures of denitrifying bacteria, this further strengthened the belief that the redox potential may serve to define a specific type of metabolism, a first and important step in the direction of a quantitative description of metabolic processes in electrochemical terms. But it soon appeared that in the case of alcoholic fermentation the measured potentials conflicted with observations made by the use of other techniques, for shortly afterwards Fromageot and Desnuelle [1935] showed that a fermenting yeast suspension can reduce dyes, such as Nile blue, whose normal potential is considerably lower than the potentials determined by Kluyster and Hoogerheide. This, of course, implied that the reducing capacity of such a suspension must be much greater than what could be surmised from the values established with the aid of noble metal electrodes.

Once again theoretical considerations of the situation provided a solution of this problem. In a subsequent publication Kluyster and Hoogerheide [1936b] argued that the potentials they had determined must be ascribed to the presence around the electrodes of a redox system of unknown composition that had diffused out of the metabolizing cells into the surrounding medium. At first the nature of this system, referred to as the 'bio-indicator', had been deemed unimportant. But now it became clear that this was not necessarily so; after all, the electrode can only measure a potential that is determined by the continuous interaction of the bio-indicator system with the electrode on the one hand, and with the intracellular catalysts on the other. The argument continues:

'Now in the previous communications we had tacitly assumed that the bio-indicators secreted by the cells would be able to display their mediating function over the entire range of potentials concerned in the metabolic activity. On closer consideration this may, however, be doubted. . . . It is by no means evident why it should be a general property of living cells to excrete into the surrounding medium an uninterrupted series of redox systems with decreasing normal potentials. Thus it is possible, nay, even probable that the oxido-reduction processes

participating in metabolism may often cause lower potentials than those measured by means of the metal electrodes in the cell suspensions.'

In this manner the apparent contradiction in the results obtained by Kluyver and Hoogerheide and by Fromageot and Desnuelle, respectively, could readily be explained. It was but necessary to assume that the dyes could interact with intracellular catalysts, representing a redox system at an even lower potential, but not excreted into the medium, and therefore unable to exert an effect on the electrodes. Consequently the potentials established at the electrodes did not necessarily represent the potentials inside the cells resulting from the particular metabolic activity under investigation. At the same time, the observed reduction of Nile blue suggested to Kluyver and Hoogerheide a simple method whereby the potential in the interior of metabolizing cells might become measurable with the aid of an external electrode. This consisted in the addition to the cell suspension of a readily diffusible redox dye of appropriate normal potential; it was felt that the dye would mediate the necessary contact between the intracellular catalysts and the electrodes. A series of measurements conducted in this manner showed that now the electrode potentials in fermenting yeast suspensions were indeed much lower than those previously determined, and fell in the range of -30 to -40 mV. It is true that some exceptions were noted; in suspensions containing certain dyes with normal potentials high enough to warrant the expectation that they should at least be partially reduced – e.g. indigo di-, tri-, and tetra sulphonates – the measured potentials were once again found to be around 100 mV. This result could, however, be attributed to an inability of these substances to penetrate into the cells, an interpretation that was supported by tests showing that such indicators are not reduced at all.

These experiments led Kluyver and Hoogerheide to introduce a general technique for the measurement of redox potentials free from the ambiguity resulting from a dependency on excreted redox systems. The technique involves measurements of electrode potentials in cell suspensions supplemented with a mixture of redox dyes covering a wide range of normal potentials, a 'universal indicator mixture'. Appropriate experiments with the various yeasts and *Ps. lindneri* then revealed that under these conditions an electrode potential of -30 to -40 mV was established in suspensions of all of them. This was therefore

considered to be the potential characteristic of alcoholic fermentation.

In similar experiments with a large variety of homofermentative lactic acid bacteria a redox potential of -160 mV was found in every case, which thus appeared to define the specific potential of a lactic acid fermentation. Here, too, some anomalies had to be faced, such as the fact that some *Streptococcus* species, although typical lactic acid bacteria in all respects, cannot reduce methylene blue, whose normal potential is surely high enough to cause its complete reduction in an environment with a redox potential as low as -160 mV. This contention was obviously supported by the rapid and complete reduction of methylene blue in cultures of other lactic acid bacteria. Furthermore, some species differ markedly from others in their ability to reduce litmus and indigocarmin, a finding that seemed incompatible with the establishment of identical potentials in cultures of all the lactic acid bacteria containing the 'universal indicator mixture'.

The experience gained in the studies on the redox potentials of micro-organisms carrying out an alcoholic fermentation led, however, to a ready explanation of these anomalies. First of all, it was found that the electrode potentials of cultures of various lactic acid bacteria not containing the indicator mixture were by no means identical, and might even differ by as much as 200–300 mV. This suggested that some species do, in fact, excrete redox systems with much lower potentials than others. Secondly, this differential behaviour also made it obvious that the latter types cannot reduce indigocarmin; this dye does not ordinarily penetrate into living cells, so that its reduction can be accomplished only under the influence of excreted redox systems at a sufficiently low potential. And thirdly, the failure of some species to reduce methylene blue could be attributed to the fact that this substance is quite toxic to these bacteria; they cannot grow and metabolize in media with the usually employed concentration of this dye, which accounts for the fact that no reduction can be observed.

Initially these studies on the redox potentials in suspensions of metabolizing micro-organisms may well have appeared to hold out promise for the subsequent development of a quantitative theory of biocatalysis, and they were extended to a number of other metabolic processes. Thus Kingma Boltjes [1935], while studying the nitrifying bacteria in Kluyver's laboratory, determined the potentials in cul-

tures of these organisms, and Roelofsen [1934], in the course of his investigations of the metabolism of the purple sulphur bacteria, measured the potentials in suspensions of a *Chromatium* species, both in darkness and when exposed to light, thereby revealing that illumination caused an instantaneous shift of the potentials to a new level, some 100 mV more positive than that of the suspension in darkness. While these measurements were of a rather incidental nature, this cannot be said of the study of Cozic [1936], who determined the potentials of suspensions of acetic acid bacteria during the oxidation of alcohol. This investigation was conducted with six different *Acetobacter* species, including *A. peroxydans* and *A. suboxydans*, thus covering the entire range of representatives with different oxidative capacities. It was found that, regardless of the species used, the potential was the same in all cases. This, no doubt, must have made Kluver realize that the measured potentials were determined exclusively by the particular metabolic process taking place in the suspensions of the microbes under investigation; and it is probable that this recognition eventually convinced him that redox potentials could not provide the requisite data to define the 'affinity for hydrogen' of different organisms, which had been the primary aim of these studies and was intended to serve as the basis for a quantitative theory of biocatalysis. This would account for the fact that after 1936 no further work on this subject appears to have been carried out in his laboratory.

That the decision to abandon this project was a sound one has become clear from the results of a quarter century's research on isolated enzyme systems. For, although current knowledge of the redox potentials of these entities has made it possible to assign to many of them a definite place in the sequence in which they participate in biochemical reactions, it is apparent that 'affinity for hydrogen' is certainly not the only factor that determines their activity. However much enzyme research may have contributed to our understanding of the detailed mechanism of biocatalysis, it has not yet led to the emergence of concepts that can explain the potentialities of various microbes to metabolize diverse types of substrates without invoking a considerable degree of arbitrariness in the distribution of specific enzymes among the organisms. Whether this will ever be feasible can only be decided by future developments.

COMPARATIVE BIOCHEMISTRY

The concept of the 'unity in biochemistry' evolved from the idea that all metabolic processes can be interpreted as sequences of step reactions, each one representing an intra- or intermolecular transfer of hydrogen under the influence of cellular catalysts. During its development several types of sugar fermentations had been analyzed in some detail, and it had been found that all of these could be explained as the end result of an identical primary conversion, leading through hexose monophosphate to methyl glyoxal, with modifications arising only in the fate of the latter compound (See, *e.g.*, Kluyver's review [1935]). It has already been mentioned that, by changing the environmental conditions during the fermentation, the intermediate products derived from methyl glyoxal could be diverted into channels that were more or less abnormal for the particular organism; the formation of acetyl methyl carbinol by yeast and lactic acid bacteria in media with added hydrogen acceptors is a good case in point. Later Kluyver and Molt [1939] showed that *B. coli* can also be induced to form this substance in trace amounts.

Of even greater significance was the application of the theory to the fermentations of substances other than the common hexoses. An excellent example is furnished by the studies of Braak [1928Th] in Kluyver's laboratory on the fermentation of glycerol; they established that the anaerobic decomposition of the polyalcohol by members of the coli-aerogenes group of bacteria may follow one of two distinct patterns. The first yields products that qualitatively and quantitatively resemble those of a sugar fermentation by the same organism; this type of fermentation is characteristic for strains that can decompose glycerol only in the presence of an additional hydrogen acceptor with which glycerol can first be converted into a triose, the latter being the genuine fermentable substrate. The second is encountered in those cases where glycerol can be fermented in the absence of an additional acceptor; these fermentations are characterized by the appearance of trimethylene glycol (1,3-propane diol) as a fermentation end product in an amount roughly equal to one-half of the glycerol fermented, the other half being recoverable in the form of products typical of a normal sugar fermentation. Such results could easily be understood by assuming an initial conversion of glycerol in which one molecule of the

substrate is oxidized with the concomitant reduction of another, according to the equation:



An essentially similar situation was encountered some years later when Barker [1936b, 1937a], during his sojourn in the Delft institute, studied the fermentation of malic, fumaric, and tartaric acids by bacteria of the coli-aerogenes group, and of glutamic acid by anaerobic spore-forming bacteria. Here, too, comparable initial conversions, such as

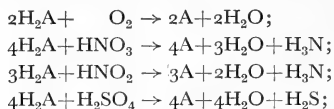


could be invoked to explain the accumulation of succinic acid, and of other products by a further degradation of oxaloacetic acid through pyruvic acid.

The fermentation of the methyl pentose, rhamnose, by *B. rhamnosifermentans* provides a good example of the ready applicability of the theory to another aberrant type of metabolism. Kluyver and Schnellen [1937] found that in this fermentation 1,2-propane diol is formed in an amount approximately corresponding to one mole per mole of rhamnose fermented. Apart from an indication in an earlier report that it may be produced in small quantities during the commercial manufacture of glycerol by fermentation of sugar in the presence of sulphite, this was the first, and so far the only record of the appearance of the asymmetrical propane diol as a major metabolic end product. Its formation was explained by postulating a rupture of rhamnose to lactic and glyceric aldehydes, the former acting as the virtually exclusive hydrogen acceptor for those reactions by which the glyceraldehyde is further degraded. The formation of succinic acid, which was found in considerable quantities in this fermentation, was, as in the previous studies of Braak [1928Th] and Scheffer [1928Th] on fermentations by members of the coli-aerogenes group, attributed to a cleavage of hexose into a two- and a four-carbon fragment; the hexose was assumed to be generated by a condensation of two molecules of glyceraldehyde. A somewhat more detailed discussion of this aspect will be found in a later section of this chapter.

Not less fruitful than in the above-mentioned cases was the application of the theory to those metabolic phenomena that can best be

described as anaerobic oxidations. These are processes in which a substrate can be oxidized under strictly anaerobic conditions with the simultaneous reduction of certain mineral constituents of the medium. Specific examples are the processes known as nitrate, nitrite, and sulphate reduction. Formerly these had been interpreted as reactions in which the reducible component of the medium supplied the oxygen necessary for the oxidation of the substrate; now they could more properly be regarded as examples of substrate dehydrogenations with nitrate, nitrite, or sulphate acting as the specific hydrogen acceptors. Thus the oxidation of a particular substrate, H_2A , could be envisaged to take place with the participation of various acceptors, and the several possibilities expressed by the over-all reaction equations:

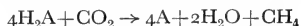


obviously all of these represent special instances of the general equation:



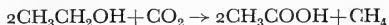
These comparisons were particularly significant because they eventually suggested an explanation for the general mechanism of the methane fermentation which Kluyver, in his London lectures, had characterized as 'the *ultimo ratio* in the domain of oxido-reduction. . . . It is obvious that the extreme form which can be conceived for such a process will be, that part of the carbon atoms present in the substrate attain their highest reduction stage, *i.e.* methane, another part of these carbon atoms their highest stage of oxidation, *i.e.* carbon dioxide' (p. 75). At that time Kluyver had to admit, however, that the 'mechanism of this type of fermentation is as yet quite unknown' (p. 76).

But a comparative-biochemical consideration of the methane fermentation as another anaerobic oxidation process later suggested that, in analogy with the nitrate, nitrite, and sulphate reduction, the methane fermentation could be interpreted as a case of substrate oxidation coupled with the reduction of carbon dioxide, a 'carbonate reduction':



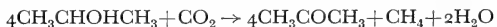
This hypothesis received strong support from Barker's [1936a] dis-

covery, in Kluyver's laboratory, of a methane fermentation in which ethanol, the only organic ingredient of the medium, was oxidized quantitatively to acetic acid, while simultaneously methane was formed, and an equivalent amount of carbon dioxide disappeared:



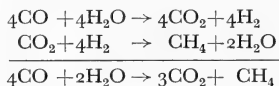
Later the origin of methane from carbon dioxide was established quite unambiguously by Barker and collaborators [1940] in experiments demonstrating that the methane formed by a pure culture of the causative bacteria during the oxidation of ethanol in the presence of $^{11}\text{CO}_2$ was, indeed, $^{11}\text{CH}_4$.

The rationale of Barker's use of ethanol as the oxidation substrate for the study of methane production had its origin in yet another application of the comparative biochemical approach. It was based on the fact that various micro-organisms oxidize this substance only as far as acetic acid; this applies, for instance, to the strictly aerobic acetic acid bacteria, notably *A. suboxydans*, as well as to particular representatives of the strictly anaerobic sulphate reducing bacteria, as had been shown by Kluyver's collaborator, Baars [1930Th], in his extensive studies of this group of organisms. The advantage of such an incomplete oxidation is, of course, that the initial substrate can still be recognized in the oxidation product, which justifies the inference that the substrate carbon cannot be implicated in the formation of methane. A comparable incomplete oxidation was later used by Kluyver and Schnellen [Kluyver, 1939a; Schnellen, 1947Th] to provide additional evidence for the origin of methane from carbon dioxide, before Barker had carried out his experiments with the radio-active carbon isotope. They succeeded in inducing a methane fermentation with isopropanol as the oxidizable substrate, and showed that the latter was quantitatively oxidized to acetone, while simultaneously carbon dioxide disappeared in an amount equivalent to that of the methane generated, according to the equation:



During their studies on the methane fermentation Kluyver and Schnellen [1947] also discovered two anaerobic bacterial species that can produce methane in an environment that contains carbon monoxide as the only oxidizable substrate [Schnellen, 1947Th]. Appro-

priate experiments led to the conclusion that the conversion of carbon monoxide by these organisms, *Methanosarcina barkeri* and *Methanobacterium formicicum*, proceeds in two stages, viz., an initial conversion of carbon monoxide and water to carbon dioxide and hydrogen, followed by a reduction of carbon dioxide with the hydrogen liberated in the first reaction; the fermentation can thus be expressed by the equations:



Ms. barkeri could accomplish this conversion in an atmosphere of pure carbon monoxide; *Mb. formicicum* appeared somewhat sensitive to this gas, tolerating it in concentrations up to about 12 per cent.

While investigating the methane fermentation in ethanol-calcium carbonate media, Barker [1937a] had also observed the formation of higher fatty acids, especially butyric and caproic acids, in those crude cultures that contained, besides the methane producing organisms, an anaerobic sporeforming bacterium. This organism was subsequently isolated in pure culture and described as *Clostridium kluyveri*; this became the starting point for the highly important studies of Barker and Stadtman on the mechanism of fatty acid synthesis.

In the meantime it had also become apparent from studies in other laboratories that carbon dioxide could no longer be considered as a mere end product of the metabolic activities of non-photosynthetic organisms acting on organic substrates. For simultaneously with the demonstration that the methane fermentation represents a process of carbon dioxide reduction, three other metabolic reactions had become known that involve carbon dioxide as one of the participating molecular species. These were:

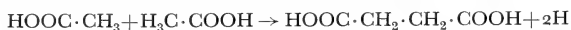
1. The synthesis of formic acid from carbon dioxide and hydrogen under the influence of *B. coli*, which Woods had shown to catalyze the reversible reaction,



This must have come as a severe shock to Kluyver who, some ten years earlier, had strenuously maintained that such a formation of formic acid, postulated by De Graaff, was too improbable to be taken seriously.

2. The formation of acetic acid from carbon dioxide and hydrogen by *Clostridium acetium*, which Wieringa [1936, 1940] had discovered during a study of the utilization of hydrogen gas by microorganisms under anaerobic conditions.
3. The assimilation of carbon dioxide during the fermentation of glycerol by propionic acid bacteria, observed by Wood and Werkman. This observation led to the recognition that many cell types can catalyze such a process in which carbon dioxide is condensed with a three-carbon compound, resulting in the formation of four-carbon dicarboxylic acids; this has become the basis on which the production of succinic acid in various fermentations is best explained. This, too, must have come as a complete surprise to Kluyver who until then had defended the thesis that the formation of succinic acid during the anaerobic decomposition of sugars and related compounds could only be attributed to an initial cleavage of hexose into a two- and a four-carbon fragment, a possibility first suggested by Virtanen. The reasoning followed by Kluyver is of sufficient general interest to warrant a brief discussion.

Kluyver started from the premise that there were only two alternative mechanisms that could account for the formation of succinic acid; in addition to the one suggested by Virtanen and mentioned above, there was the reaction proposed by Thunberg and Wieland which involved a condensation of two molecules of acetic acid with the simultaneous elimination of two hydrogen atoms:



This mode of formation had, however, been ruled out by the following argument. If the primary degradation of a hexose molecule invariably yields two triose moieties, then all products with two carbon atoms and their derivatives should be accompanied by an equimolar amount of one-carbon products which, in fermentations, are generally represented by formic acid and carbon dioxide. Now, the results of quantitative analyses of fermented sugar solutions invariably showed that, whenever succinic acid was encountered among the products, there was a marked deviation from the required ratio of two- and one-carbon products in favour of the former. This was considered sufficient to dismiss the Thunberg-Wieland mechanism as a possible source of the dicarboxylic acid.

Furthermore, these analytical data indicated that part of the two-carbon products must have originated by a different mode of sugar decomposition, which would not imply the formation of an equivalent amount of one-carbon products; and this was conveniently provided by the postulated degradation to two- and four-carbon fragments, the latter being the immediate source of the succinic acid. And because the quantitative relations between the amounts of succinic acid, two-carbon compounds, and one-carbon products closely agreed with those predicted on the basis of an assumed simultaneous occurrence of both a symmetrical and an asymmetrical cleavage of the sugar, this was consequently considered as conclusive evidence in favour of the mechanism proposed by Virtanen.

It will be obvious that this kind of reasoning is pertinent only as long as the one-carbon fragments produced in the course of the fermentation do not undergo subsequent changes by which they are converted into substances with more carbon atoms. The above-mentioned investigations of Wood and Werkman on the actual disappearance of carbon dioxide during a fermentation suffice, however, to render the above argument invalid.

However great the jolt may have been, Kluver quickly rallied to the new situation. In a lecture delivered in Helsinki in 1939 he reviewed the recent developments concerning the participation of carbon dioxide in the metabolism of heterotrophic micro-organisms and their implications; it was on this occasion that he ventured the remark:

‘Under these circumstances we may well ask . . . whether we should not make ourselves familiar with the idea that even the cells of certain animal organs can assimilate carbon dioxide.

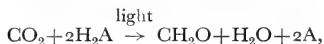
‘If at present a lecturer were to proclaim that the cattle in the pasture, nay, even the members of his audience assimilate carbon dioxide, he may expect to be showered with energetic protests. Nevertheless, the temptation to do so is certainly not easy to resist!’ (p. 86).

In this lecture Kluver also discussed certain experimental results of his collaborator, Hes, who had shown that suspensions of various micro-organisms failed to metabolize in a rigorously carbon dioxide-free environment. In trying to account for this fact Kluver considered a number of possibilities without, however, reaching a satisfactory conclusion. This is not surprising; it was not until the role of the dicarboxylic acids in oxidative metabolism, and particularly in the tri-

carboxylic acid cycle, had been clarified that a rational interpretation could be advanced.

Meanwhile Kluver's theory of metabolism had also opened the way to a re-interpretation of the mechanism of photosynthesis. In 'Die Einheit in der Biochemie' this was foreshadowed in the brief discussion of the properties of the purple sulphur bacteria. Here it was pointed out that the earlier attempts of Winogradsky, Engelmann, Molisch, and others to account for the behaviour of these organisms suffered from the fact that they had attributed the importance of light for the development of the purple bacteria to the production of oxygen by a decomposition of carbonic acid with the aid of absorbed radiant energy, even though 'no one has yet succeeded in demonstrating oxygen production under the most diverse conditions'. But once the dehydrogenation theory of biochemical reactions is accepted, this fundamental difficulty disappears because 'it is then no longer imperative to assume that the acceptor for the dehydrogenation of hydrogen sulphide or sulphur must needs be oxygen; it would be entirely possible for some other acceptor to play this role. It is merely necessary to retain the principle that the hydrogenated acceptor compound can transfer the hydrogen to carbon dioxide with the aid of radiant energy' (p. 175).

The work begun in Kluver's laboratory on the metabolism of the purple bacteria soon led to a generalized formulation* of photosynthesis as a light-dependent transfer of hydrogen to carbon dioxide with a concomitant dehydrogenation of an appropriate oxidizable substance, H_2A , according to the overall equation:



an exact counterpart of Kluver's equation expressing the metabolic processes of non-photosynthetic organisms. This formulation made it clear that oxygen evolution in photosynthesis will be observable only if H_2O is the ultimate hydrogen donor for the reduction of carbon dioxide; in all other cases a different dehydrogenation product, 'A', should make its appearance. Afterwards Muller [1933Th], on the basis of experiments concluded in Kluver's laboratory, established that organic substances may serve as hydrogen donors in purple bac-

* *Ed. note:* C. B. van Niel. Arch. Mikrobiol. 3, 1, 1931.

teria photosynthesis, and a year later Roelofsén [1934, 1935Th], in the same institute, made the important discovery that molecular hydrogen can also fulfil this function. Kluyster's continued interest in this subject has made itself felt in the many and important contributions to photosynthesis published by the 'Biophysical Group' in Utrecht, largely supported by the Rockefeller Foundation, and of which Kluyster was one of the two directors.

This same group also significantly advanced our knowledge of bioluminescence. Although several of the publications on this phenomenon deal with its physical aspects, some were clearly inspired by the comparative biochemical approach; these may here be mentioned in particular.

In a series of measurements on the relation between oxygen consumption and intensity of luminescence of suspensions of luminous bacteria under diverse conditions, it was convincingly established that specific inhibitors of respiratory activity could reduce the rate of oxygen utilization by a large factor without appreciably diminishing the amount of radiant energy emitted. This result led to the notion that bioluminescence, although characteristically an oxidative process, should be interpreted as an incidental side reaction of the normal respiratory mechanism. It also became the basis of a simple modification of Beijerinck's classical method for demonstrating the dependence of bioluminescence on the presence of oxygen, with which this point can be illustrated in a striking and elegant manner. The experiment of Beijerinck is carried out as follows: a glass tube is filled almost completely with a suspension of luminous bacteria, whereupon the open end of the tube is closed off. After standing for some time the suspension no longer emits light except at the very boundary between the liquid column and the gas phase, because the oxygen initially present in the suspension has been completely consumed. If now one turns the tube upside-down, the air passes through the liquid, which causes the entire suspension temporarily to become luminescent once more; the duration of this spectacle depends on the amount of oxygen that has gone into solution as the air bubble passes through the suspension, and on the rate of its consumption, which latter is normally determined by the density of the suspension. The experiment can be repeated many times, and will only fail if all the oxygen in the gas phase has been used up.

In the above-mentioned modification this experiment is conducted with two such tubes. When it has been ascertained that after turning the tubes upside-down the luminescence persists in both tubes for approximately the same length of time, a small amount of cyanide is added to one of the tubes, and after the suspensions have once more become non-luminous, both tubes are simultaneously inverted. The immediately observable intensity of the light emission is not perceptibly different in the two tubes; but in the tube with cyanide the luminescence lasts very much longer, demonstrating that the oxygen supply, through inhibition of the respiration, disappears at a greatly reduced rate.

Another important contribution of this group was the unequivocal demonstration with the aid of luminous bacteria that, contrary to what had been claimed by Keilin and Hartree, oxygen is not required for the decomposition of hydrogen peroxide by catalase. In a gas-tight apparatus, in which the absence of even trace amounts of oxygen could be guaranteed by virtue of the fact that a suspension of luminous bacteria failed to emit light, catalase and hydrogen peroxide were mixed. This resulted in the instantaneous reappearance of luminescence, signifying that oxygen was being produced, and hence that the enzymatic decomposition of the peroxide does not depend on the presence of free oxygen. Afterwards Keilin and Hartree found that their original contention was based on a misinterpretation of their experimental results, and that these were caused by the inactivation of the catalase by toxic nitrogen-oxygen compounds that were present in the gas they had used to render their apparatus oxygen-free.

In the preface to his lectures at Harvard University on the anatomy of science, G. N. Lewis, one of the great philosopher-scientists of this century, stated that 'the strength of science lies in its naïveté'. This also holds for Kluyver's biochemical speculations and generalizations, and he was himself fully aware of it, as is shown by his use of Lewis' phrase as the motto for the preface to his own London lectures. The attribute of strength of these biochemical concepts is amply evident from the fact that, in reducing the whole of biochemistry to a very small number of reaction patterns, a great simplification was achieved which also permitted inferences with respect to some hitherto enigmatic biochemical processes that thereby appeared in a fundamentally new

light, and thus contributed to their eventual solution. Furthermore, the comparative biochemical approach often indicated an immediate answer to some less baffling problems. A good example is furnished by the identification of a pigment produced by *Pseudomonas aureofaciens*, discovered in Kluyver's laboratory, as phenazine- α -carboxylic acid. It is significant that Kluyver [1956], in his posthumous publication on the subject, introduced the experimental part in the following words:

'It is well known that the genus *Pseudomonas* comprises several pigment-producing species, and that in all cases in which the constitution of these pigments has been established they have been found to be phenazine derivatives. . . . (Thus it seemed) worthwhile to look out for further pigment-producing species within the genus *Pseudomonas* in order to check if still other phenazine derivatives occur as natural products. The opportunity presented itself in 1936 when Mr. Bouman, working in this laboratory, incidentally came across a bacterium which was easily identified as a *Pseudomonas* species, and which attracted attention by an ample formation of yellow crystals in its colonies' (p. 406).

But the element of naïveté was not lacking either; it cannot be denied that Kluyver sometimes found it hard to resist the temptation to promulgate an explanation of certain phenomena even in the absence of an adequate body of factual information to support it; and the tendency to be satisfied with such an explanation if only it supported the concept of comparative biochemistry and the 'unitary theory' may occasionally have delayed more searching studies.

This aspect is illustrated by the studies on yeast metabolism that were intended to show that certain observations made elsewhere, although at first sight contradicting Kluyver's theory, could nevertheless be reconciled with it. The investigation of the co-zymase problem discussed earlier was a case in point; here another example may be mentioned.

After having invoked an identical series of primary reactions for the transformation of sugar into two triose moieties for the explanation of all known sugar fermentations, it was, to use a favourite phrase of his, 'tempting to postulate' that the same conversions would also be involved in the initiation of the oxidative degradation of sugars by many different organisms. This 'unitary theory' was threatened when Lunds-gaard published his studies on the effect of mono iodoacetic acid on

the fermentative and oxidative metabolism of yeast in which it had been shown that fermentation can be completely suppressed by concentrations of the poison that had no appreciable effect on the oxidative metabolism.

Kluyver and Hoogerheide [1933b], after having corroborated Lunds-gaard's experiments and determined the exact concentration of iodoacetic acid that produced the differential effect under the conditions of their experiments, then found that even the slightest raise in concentration of the inhibitor immediately affected the respiratory activity as well. This result was used to defend the thesis that the situation was fully compatible with the unitary theory. The argument was developed on the basis of the following assumptions:

1. Methyl glyoxal is the immediate substrate for both fermentation and oxidation;
2. oxidation takes precedence over fermentation, but the rate of the former is limited by the capacity of the oxygen-activating catalyst;
3. in the absence of iodoacetic acid the rate of methyl glyoxal formation greatly exceeds that of oxygen activation, so that under these conditions a considerable surplus fermentation will be observable;
- and 4. the principal effect of iodoacetic acid is that it diminishes the rate of formation of methyl glyoxal.

From these assumptions it logically followed that an inhibition of the oxidative sugar metabolism of yeast by iodoacetic acid can be expected only if its concentration has become sufficiently high to cause the rate of methyl glyoxal formation to drop to the point where it is less than the rate of oxygen activation; at lower concentrations the poison will merely cause a decrease in the rate of fermentation.

This explanation is certainly not without merit. But the above assumptions also imply that, at the concentration where iodoacetic acid just begins to inhibit the respiratory metabolism, methyl glyoxal must still be generated at a commensurate rate, so that at this concentration a comparable rate of fermentation should still be observable under anaerobic conditions. This is contrary to fact. Kluyver and Hoogerheide were well aware of this difficulty, and explained the disturbing fact by invoking the auxiliary hypothesis that, in addition to inactivating the methyl glyoxal-producing enzyme system, iodoacetic acid also causes an oxidation of reduced glutathione which latter substance would play a vital role in the fermentation reactions proper.

Similarly, the early studies by Kluyver and co-workers on the oxi-

dation of disaccharides by yeasts that cannot ferment these sugars, were, as has been mentioned earlier, largely attempts to negate the occurrence of such phenomena which were contrary to the expectations based on the unitary theory. Only at a later stage, when experiments in his own laboratory had incontrovertibly shown the reality of such occurrences, was the situation investigated in somewhat greater detail. In the publication with Custers [1940] on the suitability of disaccharides as respiration and assimilation substrates for glucose fermenting yeasts that do not ferment these sugars the problem is introduced in a manner calculated to emphasize the difference with which it had been treated by others who had recorded similar observations: 'It is true that these authors do not offer any special comments to their results' (p. 123), suggesting that they may have been unaware of the fundamental problem that was raised by such experimental findings.

When they had established that certain yeasts can oxidize a number of disaccharides which they cannot ferment, Kluver and Custers next attempted to detect the presence in such yeasts of enzymes that can hydrolyze these sugars to their constituent hexose units. The positive results of these experiments indicated that the inability of the yeasts to ferment the disaccharides could not be attributed to the absence of appropriate hydrolases *per se*. This made it unnecessary to ascribe the oxidation of such sugars to the operation of a mechanism that did not involve a preliminary hydrolysis, sometimes postulated and designated as a 'direct' oxidation of disaccharides. Since the hydrolases were present, the only way to explain the non-fermentability of the disaccharides seemed to call for the assumption that 'under anaerobic conditions these hydrolases are inactivated either completely, or at least to such an extent that the fermentability of the disaccharide is not detected by the relatively insensitive routine methods for the determination of this property' (p. 159).

Two possible reasons for the inactivity of the hydrolytic enzymes under anaerobic conditions were briefly discussed. The first one implicated a differential permeability of the disaccharides in the presence and absence of oxygen. After reviewing the results obtained by various investigators in studies on cell permeability under different conditions it became clear that in some cases permeability may be increased, in others decreased by anaerobiosis. This situation led to the following argument:

'We must, therefore, conclude that the question of the influence of oxygen on the permeability of yeast cells has not yet definitely been settled.

'Nevertheless, it is difficult to conceive that anaerobiosis should decrease the permeability of the yeast cells for the disaccharides under consideration. For these cells show a normal Pasteur-effect with glucose or well-fermentable disaccharides as fermentable substrates, proving that by withdrawal of oxygen the permeability for these sugars either remains unchanged, or even – according to Dixon's conception – is considerably increased. Now, it seems extremely improbable that the cells behave quite differently towards supposedly unfermentable disaccharides than towards the other sugars mentioned.

'On rejecting the permeability hypothesis we have to accept the second possibility, *viz.*, that the inactivation is due to a reversible change of the catalyst itself. Taking into account that withdrawal of oxygen inevitably leads to an increase in the state of reduction in the interior of the cell, it is only logical to assume that the said change is connected with a reduction process.

'Until now no evidence for the idea that carbohydrases are inactivated by reducing agents is available' (p. 160).

This statement is difficult to interpret. Does it mean that, by using this negative phraseology, the authors tried to evade another problem that could here be raised? For it is obvious that this explanation does not answer the question why these same disaccharides can readily be fermented by other yeasts under equally reducing conditions, so that it could legitimately have been said that there was proof positive to the effect that the hydrolases are not inactivated simply as a result of the absence of oxygen. Moreover, Kluyver and Custers had obtained from autolyzed yeast suspensions preparations exhibiting hydrolytic activity, and it should have been easy to test the effect of anaerobiosis and of reducing substances on the activity of the hydrolases. Nevertheless, the publication contains no evidence that such experiments were either carried out or even contemplated. These remarks suggest that, once a reasonably satisfactory explanation had been found, the problem was considered settled. It is, of course, possible that the experiments had actually been made, but, contrary to expectation, had yielded results that refuted the contention. A somewhat similar situation has previously been discussed in connexion with

Kluyver's early investigation of the assimilation of disaccharides in which it had been found that maltose could act as a satisfactory nutrient by virtue of the impurities it contained. But in that case the predicament was a fundamentally different one because the fact that purified maltose failed to induce growth could only mean that other substances were responsible for the effects and it was not necessary to identify them. In the present case the question was whether or not anaerobiosis could inhibit the activity of hydrolases of particular yeasts. Hence a negative result would have invalidated the interpretation proposed by Kluyver and Custers, and the withholding of pertinent experimental results would not have been sound scientific strategy. Therefore it seems more reasonable to conclude that the experiments were not performed.

This same publication also contains some remarks indicative of Kluyver's dissident attitude towards the concept of a 'direct' oxidation of disaccharides. It was claimed, for example, that 'it is also quite difficult to conceive that a disaccharide will undergo oxidation without preceding hydrolysis. . . . Such a "direct" oxidation . . . is further quite incompatible with the unitarian theory of respiration and fermentation' (p. 122). Again the implication seems to be that the 'unitarian theory' must be considered as the most satisfactory guide in formulating interpretations of biochemical phenomena. But in science a theory, no matter how attractive, must yield to facts, and in later years Kluyver had to admit the existence of such direct oxidations. In fact, with De Ley and Rijken [1951] he extended the earlier observations of Stodola and Lockwood on the oxidation of maltose and lactose to malto- and lactobionic acids. In a way this was not too revolutionary a discovery, for these oxidations are carried out by members of the *Pseudomonas* group with a strictly oxidative metabolism, and it was already known that at least some species of this group also oxidize glucose directly to gluconic acid. In this, as in other respects, these microbes resemble the acetic acid bacteria, and for the latter an initial non-oxidative conversion of sugar to triose had never been assumed.

Strict adherence to the unitary theory of fermentation and oxidation had to be abandoned, however, when soon afterwards it was established that the typically fermentative metabolism of some bacteria also proceeds *via* a primary oxidation of hexose sugars. By the

use of isotopically labeled glucose Gunsalus and co-workers, Gest and Lampen, and Gibbs and De Moss demonstrated that the distribution of the labeled carbon atoms of the substrate among the fermentation products arising under the influence of heterofermentative lactic acid bacteria and *Zymomonas mobilis* (*Ps. lindneri*) is entirely incompatible with the functioning of a mechanism of sugar decomposition whereby the hexose is first broken down to two triose molecules. Present information strongly supports the concept that the reaction sequence consists in an initial oxidation of the substrate to gluconic, and next to keto-gluconic acid; a decarboxylation of the latter; and finally a cleavage of the resulting pentose to a two- and three-carbon fragment.

Meanwhile De Ley had shown that the potentially fermentative *Aerobacter* species can also carry out such a direct oxidation of glucose, while Horecker and co-workers discovered a cyclic pathway for the oxidation of glucose *via* gluconic acid and pentose that now appears to be of quite general occurrence in many different kinds of cells. Needless to say, these developments have indicated that the old 'unitarian theory' can at best be applied to a very much more restricted number of cases than was at first believed.

In his second Harvard lecture Kluyver discussed in detail some of these newer findings, and introduced the startling change in outlook with the remark: 'Fortunately, however, this age has also its "sceptical biologists" ' (p. 38), concluding that review with the statement: 'Meanwhile we have learned that "there are more paths between heaven and earth" than until recently had been dreamt of in the philosophy of the comparative biochemist' (p. 45).

This sentence represents an admission of the inadequacy of the older concepts. Indubitably it has become evident that the small number of reaction patterns initially envisaged by Kluyver as constituting the whole of biochemistry must be greatly expanded if we are to comprehend the various biochemical processes in a manner more in keeping with mounting experience, and it seems a foregone conclusion that a continued study of biochemical mechanisms will reveal a number of as yet unknown types.

Kluyver had begun his search for unity in the wild variety of biochemical manifestations in 1923. In a single decade this search had produced a theory that has inspired a whole generation and guided its efforts into productive channels. Is it to be wondered at that Kluy-

ver, having experienced the powerful influence of these unsophisticated concepts, did not always succeed in immediately freeing himself of the admittedly somewhat naïve notion that they would suffice to account for all the facts?

But it is also understandable that lately the diversity has once again come to occupy the centre of attention. This has had the salutary effect of greatly expanding our factual knowledge; and, just as the multiplicity of elementary particles now accepted by physicists must sooner or later engender the desire to discover new unifying principles, so it is to be expected that in biochemistry, too, the ever increasing number of recognized reaction patterns is apt eventually to lead to the emergence of concepts from which an even more profound unity will become apparent. In the Harvard lectures Kluyver alluded to such a development in the following passage:

‘Thus we are led to the conclusion that the most fundamental character of the living state is . . . a continuous and directed movement of electrons. . . .

‘Such reflections suggest the possibility of gradually achieving an even greater simplification and unification of our views on the mechanism of metabolism than can presently be envisaged’ (p. 71–72).

This testifies to a thorough appreciation of the nature of science.

INDUSTRIAL MICROBIOLOGY AND THE SUBMERGED CULTURE METHOD FOR THE STUDY OF MOULD METABOLISM

In his inaugural address Kluyver had emphasized the potential usefulness of micro-organisms for the large-scale production of certain types of raw materials needed by the organic-chemical industry. A strong argument in its favour was the consideration that this would retard the frightening rate at which the deposits of fossil fuels were being depleted, because the microbiological processes of greatest importance in this respect are based upon the decomposition of agricultural materials, often even available as waste products. Kluyver remained keenly aware of the dangerous tendency of mankind to exploit the limited and irreplaceable natural resources of our world without paying heed to the obvious consequences. This is evident from the remarks he made in the lecture he delivered before the ‘Holland’s Society of Sciences’ under the title ‘Homo militans’:

'There is but one enemy of *Homo sapiens*, and probably the most frightening of all, whom I shall deliberately disregard; this is his brother, *Homo ignorans*. Whosoever wishes to become deeply disturbed by the manner in which, through ignorance and thoughtlessness, man himself is engaged in undermining his terrestrial existence, should read the recent book by Fairfield Osborne, "Our Plundered Planet". In this treatise, dedicated "to all who care about tomorrow", the famed President of the New York Zoological Society demonstrates how man, in consequence of the alarmingly rapid population increase, has resorted more and more to a rapacious exploitation, and thus has gradually become a geological force that even now is rapidly changing the once flourishing appearance of our planet into something akin to the desultory landscape of the moon'.

It was, therefore, not so much his belief that a Professor of Microbiology at a Technological University should concern himself primarily with the industrial application of micro-organisms on account of his position, but rather his penetrating insight into the problems of an industrial civilization, that caused him to pay close attention to the potentialities of the microbial world as purveyors of useful ingredients for industry. In connexion with the discussion of the discovery of *A. suboxydans* it has already been mentioned that he had appreciated the usefulness of the mildly and specific oxidative properties of this organism for the production of various keto-compounds, and consequently had patented this application. The same kind of foresight had been displayed in the case of the microbiological formation of 2,3-butane diol and acetyl methyl carbinol during the fermentation of carbohydrates under the influence of *Aerobacter* and *Aerobacillus* cultures. Kluyver did not fail to realize that the glycol might become an important starting material for the manufacture, by a simple dehydration process, of butadiene, which in turn is readily convertible into polyenes with rubber-like properties. The extensive studies carried out during the second world war, especially in the laboratories of the Canadian Research Council, have fully substantiated Kluyver's expectations, and it seems therefore all the more regrettable that the synthetic rubber industry has so exclusively concentrated its efforts on the production of butadiene from petroleum products, thereby causing an additional drain on the fossil fuels rather than making use of carbohydrates for the manufacture of butadiene.

Furthermore, the butane diol fermentation could also be modified so as to yield acetyl methyl carbinol instead of the glycol as a major end product. Scheffer, in Kluyver's laboratory, had shown that this can be accomplished by a proper aeration of *Aerobacter* cultures in sugar media; this apparently prevents the reduction of the primarily formed carbinol to a large extent, so that under these conditions it can accumulate in significant amounts during the fermentation. Now, acetyl methyl carbinol had been noted as a regular component of wine vinegar by Visser 't Hooft [1925a, 1925b, 1925Th] in Kluyver's institute; and he had also shown that its concentration in vinegar can be used as an excellent index of the quality of this condiment. Even more important was the fact that, in 1927, Kluyver and co-workers had identified the substance principally responsible for the characteristic and delicate flavour of high-grade butter as diacetyl, which also appears to be an important flavouring ingredient of many another food product [Kluyver, Van Niel and Derox, 1929a, 1929b]. The ready convertibility of acetyl methyl carbinol into diacetyl, either by means of a purely chemical oxidation or, even better, with the aid of *A. suboxydans*, thus emphasized the potential usefulness of the butylene glycol fermentation for several different purposes.

When, after the first decade of Kluyver's directorship of the Delft laboratory, the most important microbial sugar fermentations had been surveyed, he could begin to pay special attention to the metabolic activities of various moulds, also briefly mentioned in the inaugural address. These often lead to the production of organic acids, notably gluconic, fumaric, and citric acids, although the fundamental mechanism whereby these substances are formed were not clearly understood. It could thus be expected that a better comprehension of this type of metabolism might permit the industrial microbiologist to use conditions which improved the yield of these particular compounds, or which might be conducive to making the production of some other substances, sometimes formed in very small quantity, economically feasible. Apart from this the insight gained into metabolic reactions generally might be considerably increased by such a study of oxidative processes.

From the beginning it was evident that a commercially useful aspect of mould metabolism is that which is characterized by an incomplete oxidation of the substrate. Furthermore, the large number

of previous investigations in this field had clearly indicated that the nature of the metabolic conversion is often strikingly dependent on the conditions under which the organisms were grown. This led to the fundamental problem in how far it would be possible to differentiate between the influence of environmental conditions obtaining during growth and affecting mainly the potentialities of the cells, and of those existing during the later stages of a culture and causing a particular cell type to produce either one or another metabolic product.

Early experiments on mould metabolism had generally been conducted with growing cultures in stationary liquid media. Here the environmental conditions are subject to continuous changes, owing to the assimilation of various ingredients of the culture medium and to the accumulation of metabolic products. This had made a reasonably critical evaluation and interpretation of the results of such experiments well-nigh impossible. Besides, with such cultures it could not be ascertained whether a particular product was derived directly from the substrate provided, or might have originated by a very different, round-about route. The reason for this is that growth implies the formation of new cell materials, including reserve products, so that the latter, rather than the substrate itself, might be the direct precursors of the metabolic products in question. The formation of kojic acid by the mould, *Aspergillus flavus*, is a case in point. It is easy to envisage its formation from glucose; the closely similar constitution of the two substances suggests that the conversion would consist in a partial oxidation and dehydration of the sugar. But the same mould had also been reported to produce kojic acid when grown at the expense of various other substrates, such as pentoses, erythritol, and glycerol; and this is rather difficult to understand as the result of a direct degradation because their constitution does not at all resemble that of the product. It seemed therefore much more reasonable to assume that in these cases the substrates are first converted into reserve materials that subsequently can yield glucose, and that the kojic acid is actually formed only from this substance.

In order to test this hypothesis Kluyver and Perquin [1933b] prepared a culture of *Asp. flavus* in the manner described in more detail below, and exposed aliquots to solutions of glucose, fructose, galactose, arabinose, xylose, mannitol, erythritol, and glycerol. Analysis of the

cultures showed that, except in the glucose solution, kojic acid was not produced, thus supporting the contention that the positive results obtained by others with growing cultures were due to substrate transformations via glucose, and involving growth of the mould. It should, however, be mentioned that this experiment does not provide conclusive evidence in favour of the proposed interpretation; it is at least possible that mycelia grown in the presence of other substrates might have yielded different results.

But the main point of this discussion is to illustrate the fundamental difficulties inherent in the use of growing cultures for the study of mould metabolism. It was therefore a distinct improvement when the method of using pre-grown mycelium was introduced. This could first be freed of the initial medium by washing, and then tested for its effect on specific substrates under much better controlled conditions. Nevertheless, even this technique had been found far from satisfactory; this follows clearly from the fact that it is virtually impossible to duplicate the results of such experiments.

It was the keen recognition of the complex situation encountered in cultures of filamentous fungi that led Kluver and Perquin [1933a] to develop a new methodology for the study of mould metabolism; this was published in 1933 under the name of 'agitated cultures'. The arguments for abandoning the stationary cultures, even those with preformed mycelial mats, have been set forth so clearly, and are so convincing that they are here quoted in some detail.

Having raised the question what factors are responsible for the oftentimes incomplete oxidations performed by moulds, Kluver and Perquin concluded:

'It is clear that *a priori* two different reasons could be held responsible for this behaviour. Firstly, it is conceivable that the cells are characterized by a specific low oxidative capacity. This is true, for example, in the case of *A. suboxydans*, whose oxidative activity towards certain substrates is restricted to a single dehydrogenation, even under widely different external conditions. But secondly, it has long been known that in many cases an incomplete oxidation is determined by outside influences, so that cells fully capable of performing a complete substrate oxidation may, under special circumstances, cause the accumulation of incomplete oxidation products. For such materials

Duclaux has introduced the suggestive term, "products of suffering" (p. 69).*

Now, the experiments of Molliard had shown that the mould, *Asp. niger*, which can oxidize sugar without causing the accumulation of any acid whatever, may be induced to form considerable quantities of gluconic, citric, or oxalic acid by growing it in a culture medium with drastically reduced nitrogen, phosphate, or potassium content. 'Consequently', continued Kluyver and Perquin, 'it was obvious that for this mould at least the industrially important acid production is not a physiological, but rather a pathological process. Viewed in this light the inadequacy of the methods hitherto used in the study of mould metabolism hardly needs commentary. It must be evident how utterly heterogeneous are the conditions under which the individual cells exist in a mycelial pad growing on the surface of a liquid culture medium. Whereas the cells in the upper layers are bathed in the oxygen of the atmosphere, they must depend for their supply of nutrients on the diffusion of the latter through the mycelium, and only that fraction which is not absorbed by the cells lower down will reach them. Conversely, the latter type of cells swim in a substrate-rich environment, but have access to very limited amounts of oxygen because this gas is largely consumed in the upper layers. Between these two extremes all possible intermediate stages exist. It must be realized that part of the cells may be so poorly nourished that they become considerably weakened, and may even undergo autolysis.

'In connexion with results such as those of Molliard's this implies that the metabolism of the individual cells in the mycelium will exhibit pronounced mutual differences, and that the customary analysis of the over-all processes in such a liquid culture can only provide information concerning the combined results of the diverse metabolic processes that proceed under the influence of the various cell types.

'If one further keeps in mind that the structure of the mycelial mat

* After the discovery of *A. suboxydans* with its intrinsically low oxidative capacity, the transient accumulation of incomplete oxidation products in liquid cultures of *A. xylinum* was attributed by Visser 't Hooft [1925] to the poor oxygen provision underneath the thick pellicle which is the characteristic growth form of this organism. This notion is supported by the fact that on glucose-calcium carbonate-agar plates *A. xylinum* does not even produce detectible amounts of acid. Kluyver used to refer to this bacterium as a 'physically conditioned sorbose bacterium', in contrast to the 'ideal sorbose bacterium' which is, of course, *A. suboxydans*.

— either adhering closely to the surface of the medium, or forming more or less pronounced and raised folds — can often be greatly modified by factors that are difficult to control, then it is no longer surprising that different results are so often obtained in duplicate cultures under presumably identical conditions. But even if such cultures were to show satisfactory agreement it is still impossible to specify sharply the conditions responsible for a particular direction of the metabolic processes. If, for example, a particular situation leads to the formation of large amounts of citric acid, it is impossible to decide which of the cells in the mycelium, developing under heterogeneous conditions, have been responsible for the acid production. And even if it were possible to determine which cells have produced the acid, and to specify the optimum conditions for this process, the result would still be of only limited significance, because it would be erroneous to conclude that any cell of the organism would display the metabolic activities characteristic of the acid production under these same optimum conditions. One can find many examples in the literature showing that the metabolism of mould cells is greatly dependent on the conditions under which the cells were grown, and we shall presently add further proof for this statement. Now it is obvious that these conditions are quite heterogeneous for the cells developing in a mycelial mat on a liquid medium. In summary we may therefore conclude that one has to reckon with the presence in such a mat of cells of heterogeneous origin, exposed to heterogeneous conditions. It will then be clear that one can obtain at best a very restricted picture of the metabolic activities of moulds with the aid of the usual stationary cultures. Consequently a deeper penetration into the problems of this metabolism is possible only if it is investigated with cell material of homogeneous composition, studied under homogeneous conditions' (p. 70).

This, of course, raised the question how such cell material of homogeneous composition could be procured. At the outset it seemed possible that this might be accomplished by growing the mould so that it would develop submerged in a culture liquid rather than on top of it. This would necessitate aeration of the culture in order to supply the cells with an adequate amount of oxygen. After testing a number of different arrangements the most satisfactory results were obtained by inoculating a liquid medium with mould spores, and placing the culture on a shaking apparatus, thus maintaining the contents in a

state of continuous and violent agitation. This completely prevented pellicle formation and at the same time assured a plentiful oxygen supply. In such cultures the mould developed in the form of small balls, of uniform size, composed of tangled threads of cells that had originated under conditions as nearly homogeneous as possible. The balls of mycelium could be conveniently handled; a simple filtration through ordinary filter paper sufficed to collect and wash them free of adhering medium. Thereafter uniform suspensions could be prepared from the residue on the filter paper, permitting experiments to be conducted with a rigorous control of factors, varied one at a time.

Application of this technique to problems of kojic, gluconic, and citric acid formation showed that the results were reproducible to a remarkable degree. This also led to a better specification of the optimum conditions for the production of these substances, although it contributed little to advancing our understanding of the mechanism whereby they are generated.

The significance of this new methodology is difficult to overestimate. Foster, in his book 'Chemical Activities of Fungi', has paid due credit to it in the passage:

'From much of the foregoing it is evident that for the most effective studies on mold physiology and biochemistry, none of the methods already described are completely adequate. Submerged growth cultures theoretically and practically afford the closest approach to the ideal method of studying mold metabolism. The principles involved in the technique, and the implications possible for the interpretation of physiological studies with fungi under different conditions were first clearly enunciated in 1933 in a classic paper . . . by Professor A. J. Kluyver and his student and collaborator, L. H. C. Perquin. This and subsequent papers by these authors represent the first attempt to study mold metabolism systematically under strictly controlled conditions, and to elucidate some fundamental principles obtained thereby' (p. 51).

Those who are familiar with current practices in the industrial production of penicillin, streptomycin, and other substances formed by filamentous microbes will realize how completely this method has been adopted in microbiological practice. But that this method was devised some twenty five years ago in Kluyver's laboratory, and on purely theoretical grounds, is insufficiently appreciated.

THE CLASSIFICATION OF MICRO-ORGANISMS

In the inaugural address Kluver had also stressed the need for an intensive study of micro-organisms in various directions as a preliminary to developing a more satisfactory system of classification than those in use at the time. 'It is well known', he had asserted, 'that even to-day there exists an exasperating confusion in the area of the classification of the *Schizomycetes*'. Although the confusion was all too evident from the mere fact that several conflicting and uncoordinated systems were in use side by side, it is doubtful whether Kluver could have indicated in what respects these systems were defective, or how they could be improved. For it must be realized that a good systematist must above all be thoroughly familiar with the material to be classified, and this requires an extensive first-hand knowledge and experience such as Kluver certainly did not yet possess. But through the isolation of numerous pure cultures of diverse micro-organisms and the study of their characteristics in his laboratory this deficiency was gradually repaired. And together with his amazing memory and acute sense of order, the experience gained soon helped him to acquire the background necessary for making constructive proposals.

The chief reason for the confused state of bacterial classification was that bacteriologists with divergent aims and fields of interest had not been able to agree on what properties should constitute acceptable criteria for the segregation of taxonomic categories. As is only natural, each one had brought his own experience and preferences to bear on the problem. But these were often so different that several independent approaches had been advocated, each one supported by one or more specialists, and leading to several almost 'private' systems.

Differentiation on the basis of morphology had long ago been used by Ferdinand Cohn as a means of classifying the bacteria known to him in a few 'form genera'. In due course some additional morphological features, such as flagellation, spore formation, and staining properties, had permitted a much needed expansion of the number of these entities. Nevertheless, it soon became clear that the genera comprised types whose further differentiation was desirable, and this could be accomplished only on the basis of non-morphological criteria. Hence ecology, pathogenicity, and specific biochemical and serological properties had been introduced as additional aids in distin-

guishing pure cultures of bacteria one from another. Usually, however, the identification of a culture did not seem to require the determination of more than a few of such characteristics, and this frequently caused all others to be neglected. As a consequence one and the same organism might be recognized by one specialist on account of its appearance and mode of growth in various culture media, by another because of its effects on experimental animals, by yet others on the basis of the occurrence of particular chemical changes observed in certain environments. The result was that many bacterial cultures were only partially characterized, and not always by means of the same criteria, so that a single type might even be described under a variety of names.

As long as the total number of known bacterial types was relatively small, the few morphologically characterized groups, the 'form genera' established in the early days of bacteriological research, sufficed as a primary basis for descriptive and nomenclatorial purposes. However, the rapid increase in knowledge eventually led to the practice of combining groups of bacteria into genera that were no longer demarcated primarily or exclusively with reference to morphological features. Especially Winogradsky and Beijerinck introduced many 'genera' of this sort; *Nitrosomonas*, *Nitrobacter*, *Acetobacter*, *Azotobacter*, *Aerobacter*, *Photobacterium*, *Thiobacillus*, and *Lactobacillus* may be mentioned as examples. Now, the great advantage of such genera is that they effectively speed up identification of a newly isolated strain; any bacterium that lives in an alcohol-containing medium and produces acetic acid therein immediately becomes recognizable as an *Acetobacter* species; a luminous bacterium is, *ipso facto*, a *Photobacterium*; or a non-sporeforming nitrogen fixing bacterium an *Azotobacter*. But the practice of originating such genera also had some distinct disadvantages. For example, neither Winogradsky nor Beijerinck had ever taken the trouble to explain his reasons for proposing such genera, probably because they had considered them to be self-evident. As a consequence the undesirable implications of the procedure became clear only at a later date.

Was it sound practice to include physiological properties amongst the diagnostic features of a genus? As early as 1872 Cohn had discussed this problem in connexion with the differentiation of species within his form genera, and warned of the dangers inherent in this

practice. His negative attitude was based on a simple and convincing argument. Consider, he had said, two almond trees, one producing sweet, the other one bitter almonds. Obviously these two specimens exhibit a physiological difference that is as striking as it is constant. Nevertheless, no botanist would ever be induced to regard these trees as different species. And what guarantee was there that physiological differences between two types of bacteria were any more significant than those exhibited by the two almond trees? Now if, for these reasons, it appeared ill-advised to use physiological characteristics for the differentiation of species, how much more forcibly would the argument apply to Winogradsky's and Beijerinck's genera! Furthermore, one might legitimately ask whether such differences were constant. Also in this respect the dangers were certainly not imaginary; it need but be pointed out that Beijerinck himself had shown how easily a strain of luminous bacteria may permanently lose its ability to emit light; this happens, for example, if it is cultivated at moderately high temperatures, where growth is not adversely affected. This implies that such a modified strain would henceforth have to be identified as a member of a different genus!

Despite this criticism many of the above-mentioned genera have been perpetuated, largely because they proved to be so eminently useful. No doubt this argues strongly for the thesis that Winogradsky and Beijerinck both had a highly developed 'taxonomic intuition'. Later studies have shown that many of their genera represent assemblages of organisms with a considerable number of common, more or less basic properties. And spurred on by the example set by the two great general microbiologists, the tendency to employ physiological characteristics in bacterial systematics gradually spread. In view of the paucity of morphological properties and the obvious need for more refined supplementary means of differentiation, this was inevitable. The most elaborate and consistent use of generic names for groups of bacteria with similar physiological properties was made by Orla-Jensen in his attempt to construct a system of bacterial classification that was purported to reflect the phylogenetic relationships of these microbes. He began by recognizing two morphologically distinct lines, represented by the orders *Cephalotrichinae* and *Peritrichinae*, comprising the bacteria with polar and peritrichous flagella, respectively; non-motile bacteria were, somewhat arbitrarily, assigned to one or the

other of these groups. The subdivision of the two orders into genera was based primarily on physiological criteria, and the genera, including many new ones, were therefore largely physiologically defined. The new names proposed were designed to express directly the main morphological and physiological attributes of their members.

Although the approach had much to recommend it, the increased use of physiological properties also led to developments that may be deemed less satisfactory. The medically oriented bacteriologists now felt justified, and I believe rightly so, in regarding pathogenicity as a differential character on a par with certain biochemical ones. Eventually this led to the creation of special genera for disease-producing bacteria, and these sometimes overlapped with genera of non-pathogenic organisms. For the development of a consistent system of classification it therefore became necessary to assess the relative values of several unrelated physiological properties, and, in the absence of acknowledged standards, it usually proved impossible to achieve concordance amongst specialists of different persuasion. Another unfortunate result of the application of physiological properties for the creation of taxa of higher order was that the extremely limited comprehension of what constitutes the fundamental characteristics often led to the use of what later could be regarded as very minor qualities.

Now, as mentioned earlier, the discovery of *Acetobacter suboxydans*, and the comparison of its properties with those of other acetic acid bacteria, provided the opening wedge for Kluyver's entrance in the field of bacterial taxonomy. The very appearance of cultures of this organism on sugar-calcium carbonate-agar plates left no doubt that it represented a hitherto unknown type, and the biochemical studies had shown that it could be characterized by its low oxidative capacity. Because the other representatives of the acetic acid bacteria also seemed to exhibit differences in this respect, it had become possible to arrange all of them in order of their oxidizing ability, and to assign to those with clearly recognizable differences the status of a species or species group. This approach could also be applied readily to some strains of acetic acid bacteria that Visser 't Hooft had isolated from ditch water, through elective cultures in an acidic mineral medium with alcohol as organic substrate. These strains differed from all other *Acetobacter* cultures in being catalase negative and able to oxidize molecular hydrogen. At the time this latter feature suggested that they

possessed an exceptionally high affinity for hydrogen, which was equivalent to having a high oxidative capacity, a supposition that was further supported by the fact that they did not produce acid from glucose, apparently causing a complete oxidation of this substrate without the temporary accumulation of incomplete oxidation products. These organisms, for obvious reasons, became known as *Acetobacter peroxydans*.

The studies on the acetic acid bacteria had therefore shown that biochemical properties could be advantageously used for the subdivision of a genus already partially characterized on the basis of physiological criteria. Hence it is understandable that, when the fundamental principles of biochemistry had begun to emerge, Kluyver expected that they would be even more efficacious for the classification of micro-organisms in a still larger framework, promising that ultimately such a classification might become based on numerically determinable differences in the affinity of the protoplasm of various microbes for hydrogen. Pending the establishment of appropriate methods for the quantitative evaluation of this property, one could begin by using in its place the general metabolic patterns of the organisms which could be ascertained by relatively simple analytical procedures.

This meant that biochemical characteristics far more fundamental than, for example, the ability to ferment one or more particular sugars, would come to serve as the basis for the creation of physiologically homogeneous entities, comparable in many respects with Cohn's 'form genera'. It remained to develop a scheme in which the old form genera and the new physiological groups could be combined, so that satisfactorily circumscribed genera would ensue. That morphological and physiological features could be conveniently merged had already been shown by Donker's analysis of the fermentation pattern of *Bac. polymyxa* which had indicated the striking similarity in physiological respect of this morphologically typical sporeformer and the non-sporeforming *Aerobacter* species. By proposing the new genus, *Aerobacillus*, for the former, the feasibility of this kind of approach had been sufficiently demonstrated.

Meanwhile the study of the propionic acid bacteria in Kluyver's laboratory had shown that these organisms represent a remarkably homogeneous group with respect to both their general morphological and biochemical properties. This justified the resurrection of Orla-

Jensen's previously proposed genus, *Propionibacterium*. But this investigation had also indicated a close morphological resemblance between these organisms and several other types of bacteria, such as the lactic acid-, the coryne-, and the mycobacteria. All these organisms can be characterized as non-sporeforming, Gram-positive, and permanently immotile bacteria. This in turn had suggested the possibility of establishing, in addition to the two major morphological groups recognized by Orla-Jensen, a third one for these immotile organisms.

These developments resulted, in 1936, in a fairly elaborate attempt to formulate the basic ideas on the classification of bacteria that had gradually taken shape. The timing was shrewd; at the International Congress for Microbiology scheduled to meet in London later during that year, the International Committee for Nomenclature and Classification of which Kluver was a member would thus be in a position to take cognizance of the proposals, elaborated if necessary by Kluver himself. Because the paper has been reprinted in this volume it is not necessary to discuss it in detail; suffice it to say that it was felt to represent a first step in organizing the *Eubacteria* in a manner somewhat comparable to that in which Mendelejeff had arranged the chemical elements, and with the same advantage of indicating the potential existence of groups of bacteria with combined morphological and physiological properties not as yet encountered; if discovered, these could immediately be incorporated in the system in their appropriate places. The recent discovery of polarly flagellated bacteria with a fermentation pattern like that of the coli-group of organisms, and for which Asai *et al.* [1956] have aptly proposed the generic name, *Kluvera*, testifies to the usefulness of the approach. So do several generic names, created since 1936, and based on the same principles, such as, for example, *Methanobacterium* and *Butyribacterium*.

The simple guidelines employed in 1936 were adequate to achieve a reasonably satisfactory subdivision of those bacteria that are characterized by an obligatorily or facultatively fermentative metabolism, because it is easy to distinguish between the diverse and specific fermentation patterns. But a subdivision of bacteria with a strictly oxidative type of metabolism could not then be accomplished by a comparable approach; hence the corresponding genera were less satisfactorily defined. Much has meanwhile been learned about patterns of oxidative metabolism which suggests that an imaginative use of this in-

formation may permit the establishment of subgroups more nearly equivalent to those based on particular anaerobic processes.

Although the outline of bacterial classification presented in 1936, like any and all other systems that have thus far been proposed, is subject to the criticism that it does not rest on a truly phylogenetic basis [Van Niel 1955], its rational features have had a beneficial influence on systematic bacteriology generally, and many of the genera there delineated have gradually been incorporated in the current systems.

Besides the main outline mentioned above, the contributions to bacterial classification of Kluver and his associates comprise many publications on problems of a more limited scope. The monographic treatments of the genera *Acetobacter* [Visser 't Hooft, 1925Th; Fraiteur, 1950], *Protaminobacter* [Den Dooren de Jong, 1926Th, 1927], *Propionibacterium* [Van Niel, 1928Th], *Pediococcus* [Mees, 1934Th], and *Spirillum* [Giesberger, 1936Th]; of the group of sulphate reducing bacteria [Baars, 1930Th], brine bacteria [Hof, 1935Th], and methane producing bacteria [Schnellen, 1947Th], all these will long remain amongst the indispensable references to these groups. Giesberger later extended his studies of the spirilla to the reddish-brown members of the genus *Rhodospirillum*; Kingma Boltjes [1934Th, 1936] made a careful investigation of the nitrifying bacteria during which he rediscovered *Hyphomicrobium vulgare*, whose strange life cycle, including the multiplication by budding, was described in detail; Mayer [1938Th] and Perquin [1939] made notable contributions to our knowledge of the dextran-producing *Betabacterium vermiforme* and *Streptobacterium dextranicum*, respectively; and Kaars Sijpesteijn [1948Th, 1949, 1951] to that of the cellulose-decomposing bacteria in rumen, leading to the establishment of the new genus, *Ruminococcus*. Here may also be mentioned the discovery of *Pseudomonas beijerinckii* [Kluver and Hof, 1939; Kluver, Hof and Boezaardt, 1939], characterized by the production of a purple pigment only if the organism is grown in the presence of inositol, which is oxidized to tetra hydroxyquinone, whose calcium and magnesium complexes are responsible for the colour of the cultures; of *Ps. aureofaciens* [Kluver, 1956]; and of *Hydrogenomonas carboxydovorans* [Kistner, 1953, 1954] which can live at the expense of the oxidation of carbon monoxide. The comparative studies of the aerobic nitrogen fixing bacteria of the *Azotobacter* [Kluver and Van Reenen, 1933; Kluver and Van den Bout, 1936] and *Beijerinckia*

[Kluyver and Becking, 1955] groups, and of the nitrate-reducing sporeforming bacteria [Verhoeven, 1950, 1952Th] contain valuable information relating bacterial taxonomy to ecology; to this category may be added Elazari-Volcani's [1939] investigation of *Ps. indigofera*.

Kluyver's contributions to classification are not restricted to the bacterial kingdom, however. Through his early studies with yeasts he maintained a profound interest in this group of organisms. He had inherited Beijerinck's culture collection which included many yeasts, and soon after assuming his duties as Professor of Microbiology Kluyver arranged with Professor Johanna Westerdijk, Director of the renowned 'Central Bureau of Fungus Cultures' * in Baarn, Holland, to have the yeasts represented in the Baarn collection transferred to the Delft institute. Thus was established what is probably the world's most outstanding collection of yeast pure cultures. It has served as the basis for a series of monographs on the yeasts [Stelling-Dekker, 1931Th; Lodder, 1934Th; Diddens and Lodder, 1942; Lodder and Kreger-van Rij, 1952] comprising the best so far published in this field. The principles guiding the development of an up-to-date system of yeast classification, adopted in these treatises, can be found in Kluyver's publication of 1931 which has also been included in this volume.

Before the first of these monographs had appeared, Kluyver's extensive knowledge of microbiological literature and his keen scientific insight had permitted him to recognize the curious phenomenon of mirror-image formation by certain pink yeasts when cultivated on solid media in an inverted position as associated with a mechanism of spore discharge that is characteristically encountered among the basidiomycetes. This suggested that, contrary to the universally held opinion, not all yeasts should be considered as primitive or reduced ascomycetes, but rather that the basidiomycetes also embrace a fully comparable series of progressively more complex fungi whose simplest representatives are the typical yeasts for which the genus *Sporobolomyces* was created [Kluyver and Van Niel, 1925, 1927].

Similarly, Kluyver's wide experience and microbiological acuity were responsible for the recognition that organisms described by certain specialists as new yeast species or even genera, were, in reality,

* A note on the history of the Central Bureau of Fungus Cultures has been published by K. B. Raper in *Mycologia* 49, 884 [1957].

members of the group of aerobic sporeforming bacteria; this applies, for instance, to the species, *Schizosaccharomyces hominis*, [Dorrepaal, 1930], and to the genus, *Schizotorulopsis* [Verkaik, 1931].

Besides these contributions should be noted the monographs dealing with the yeast genus, *Brettanomyces* [Custers, 1940Th], and with the species, *Candida pulcherrima* [van der Walt, 1952Th]. The latter contains the fascinating observations on the pigments produced by this yeast in the presence of various heavy metals. This study eventually led Kluyver and co-workers [1953] to the isolation and characterization of pulcherrimin, surely one of the most interesting chelating agents known to-day.

VARIABILITY OF MICRO-ORGANISMS

From the preceding section it will be clear that Kluyver knew micro-organisms as more than entities performing special biochemical transformations, which implies that he was also familiar with that bane of the systematist that can conveniently be described by the collective term, 'variability'.

Variability expresses itself in physiological as well as morphological respects. Both of these have been studied in Kluyver's institute, and some of the contributions to this aspect of microbial behaviour will here be briefly reviewed.

In 1925 Elion [1925] had there discovered a bacterial sulphate reduction at temperatures as high as 65°C; the causative agent had been isolated and described as *Vibrio thermodesulfuricans*; it was the third species of sulphate-reducing bacteria, the previously known ones being the mesophilic freshwater *V. desulfuricans* and the marine *V. aestuarii*. The isolation of *V. thermodesulfuricans*, unable to grow at temperatures below 30°C, presented a curious problem, however. The cultures had been started with an inoculum of mud taken from a ditch below a heavy layer of ice, and it seemed most improbable that even during warm seasons the temperature of the mud in this ditch would ever reach the minimum temperature required for growth of the new species. This raised the question how its apparently regular occurrence in this environment could be explained.

Some years later this situation led Kluyver and Baars to the idea that *V. thermodesulfuricans* might not be an autonomous species, but

rather a modified form of the mesophilic *V. desulfuricans*. By using large inocula of a pure culture of the latter, and incubating transfers at increasingly elevated temperatures, it proved indeed possible to effect a change in the properties of the organism which appeared to involve a gradual shift in the entire temperature range within which the strain could develop. In like manner the reverse was also accomplished, and a thermophilic culture changed into a mesophilic one.

These experimentally induced modifications strongly supported the view that *V. desulfuricans* and *V. thermodesulfuricans* represented more or less stabilized races of one and the same organism rather than distinct and separate species. This further led to experiments designed to determine whether *V. desulfuricans* and *V. aestuarii* might be similarly related. Hitherto these species had been distinguished solely because the former can develop normally in media with 0–1.5 per cent sodium chloride, but is inhibited by higher concentrations, and unable to grow with more than 2.5 per cent, whereas the latter grows equally rapidly in media with 1–3 per cent, and not at all with less than 0.5 per cent salt. By transferring cultures to media with different salt content it was found that the range of salt concentrations at which uninhibited growth can occur is just as little fixed as is the temperature range, and that these two types can also be gradually interconverted. Consequently it was proposed to recognize only *V. desulfuricans* as a taxonomically sound species; the others were graphically designated as 'physiological artefacts' [Kluyver and Baars, 1932; Baars, 1930Th].

The thermophilic nature of Elion's isolate could be more easily understood when Starkey [1938], working in Kluyver's laboratory, discovered that sulphate reduction at elevated temperatures is caused by a sporeforming bacterium. At the time this was considered to be identical with the 'adapted' mesophilic cultures of Baars, and designated as *Sporovibrio*. But recent studies have shown that the experimental results of Kluyver and Baars are not reproducible. Eventually this situation has led Campbell *et al* [1957] to identify Starkey's *Sporovibrio* as *Clostridium nigrificans*, a sporeforming anaerobe that had long been known as the causative agent of hydrogen sulphide production in certain canned products. This conclusion has lately been corroborated in Kluyver's laboratory as well as by Starkey. It therefore seems probable that the earlier results must be attributed to the use of impure cultures.

Interconversion of freshwater and marine strains of *Desulfovibrio*

desulfuricans has also been reinvestigated elsewhere, and it has been found that strains of varied origin display unpredictable differences in behaviour in this respect. Some strains appear to be readily adaptable, others not at all [Littlewood and Postgate, 1957].

While thus the variability of some physiological properties of the sulphate reducing bacteria appears to be less general or pronounced than was claimed in 1930, the studies of Kluyver and Manten [1942] with a culture of an hydrogen-oxidizing bacterium, identified as *Hydrogenomonas flava*, provided an example of another sort of physiological variability. By means of manometric experiments it was established that suspensions of this organism grown in mineral media at the expense of hydrogen oxidation, can oxidize hydrogen gas as well as organic substrates, and that such oxidations can even proceed simultaneously; whereas cultures grown in media with organic compounds in the absence of hydrogen completely failed to oxidize the latter. This indicated that 'obviously the formation of the special hydrogen oxidizing system only takes place when the bacteria are grown under autotrophic conditions'. Such a behaviour is reminiscent of that displayed by certain yeasts with respect to their ability to ferment galactose, and, as mentioned earlier, Kluyver was fully cognizant of its significance.

The experiments with *H. flava* revealed furthermore that, after prolonged cultivation on organic media in the absence of gaseous hydrogen, its ability to grow autotrophically was irretrievably lost. A fully satisfactory explanation of this phenomenon, previously also observed by Grohmann, has not yet been proposed.

This investigation of *H. flava*, together with renewed studies in Kluyver's institute on bacterial denitrification, eventually led to a search for naturally occurring micro-organisms that can oxidize hydrogen with nitrate as the sole oxidant. The existence of such organisms was probable on theoretical grounds, but had never been conclusively demonstrated. By means of appropriate elective cultures the presence of such specialists in soil samples was readily ascertained, although it appeared that the bacteria that can carry out this conversion can grow in an anaerobic environment with hydrogen and nitrate only if a small amount of yeast extract is present in the medium.

A careful examination of such cultures showed that they contained only one predominant bacterial type which closely resembled the

denitrifying bacterium that Beijerinck had previously and rather cursorily described under the name *Micrococcus denitrificans*; the latter was not known to be a potential hydrogen bacterium, however. Just as *H. flava*, the new isolates could oxidize hydrogen as well as organic compounds, and hydrogen was not oxidized by cultures grown in organic media. Furthermore, these strains could use either oxygen or nitrate as oxidant, although nitrate reduction was exhibited only by cultures grown anaerobically in the presence of nitrate, a behaviour that is also characteristic of other denitrifying bacteria. Once these facts had been established, it was possible to test the suspicion that the new isolates were indeed identical with *M. denitrificans*, and this was confirmed when it was found that Beijerinck's original culture of that organism, maintained for many years in the Delft culture collection on routine organic media, could still be induced to form a nitrate-reducing as well as a hydrogen-oxidizing enzyme system when cultivated under the requisite conditions. In contrast to *H. flava*, *M. denitrificans* evidently retains its potentiality to live as a hydrogen bacterium even after prolonged growth on organic media.

In his Harvard lectures Kluyver referred to these studies as examples of microbial variability due to enzymatic adaptation, or, to use the current term, to induced enzyme formation, and stated:

'... it seems warranted to conclude that *M. denitrificans* offers a striking example of life's flexibility. It can live as a heterotrophe, depending on the oxidation of some organic substrate, not only with free oxygen, but also with nitrate as a substitute for the latter; next it turns out to be a fully autotrophic organism able to thrive on the Knallgas system; and finally it emerges as a chemometatrophic organism displaying the seemingly exceptional quality of thriving on the system molecular hydrogen-nitrate. But it has been clearly shown that the ability to use hydrogen as donor and nitrate as acceptor depends on the presence of special enzymes which are produced only in response to well-defined conditions during growth.

'How strongly the potentiality to produce these enzymes is fixed in the genetic apparatus of the organism is demonstrated in a most convincing way by the behaviour of Beijerinck's original culture, which, maintained for forty years – that is, for thousands of generations – on peptone agar, on being transferred to an environment where molecular hydrogen is the only energy source available, answers nature's

challenge by the brave device: "Here I am, I can also act differently!"' (p. 107).

But the microbial world also offers many examples of a kind of variability that is not primarily induced or controlled by environmental conditions. A fairly common sort is what eventually became known as 'dissociation'; it manifests itself through the appearance of different types of colonies on one and the same culture plate, streaked with a suspension of a pure culture of some micro-organism. Special terms and abbreviations, such as 'smooth' ('S'), 'rough' ('R'), 'mucoid' ('M'), etc., had been introduced to characterize various colony types. The reality of this phenomenon had been established beyond question, but a generally accepted interpretation was lacking even as late as the middle 'thirties.

As early as 1900, and again in 1912, long before the name 'dissociation' had been introduced, Beijerinck had described several cases of variability of this kind, and claimed that they represented mutations in the sense of Hugo de Vries, regularly occurring in cultures of micro-organisms. This concept had, however, been sharply contested, the opponents arguing that mutations can be observed only in organisms that reproduce sexually. Such a mode of reproduction was unknown amongst the bacteria, which were not even considered to possess a nucleus. Consequently Beijerinck's interpretation of microbial variability had fallen into disrepute.

Another theory to account for the dissociation phenomenon and which had gained prominence during the nineteen-thirties, had been developed by Hadley. It was based on the assumption that bacterial cultures regularly display ontogenetic variations, and that different 'cyclostages' are recognizable by the specific colony types corresponding to them, the 'rough' phase representing the culminating, or 'reproductively mature' stage through which every species would have to pass in due course. Nevertheless, this concept smacked too much of the 'life cycle' theories of Löhnis and Enderlein, formulated in order to account for morphological variations which others, with good reasons, had attributed to the use of impure cultures; and Hadley's speculations were not regarded very sympathetically by most of the leading microbiologists. It is clear, therefore, that the situation was confused and that further experiments were needed to resolve it.

The opportunity to carry out such an investigation in Kluyver's laboratory presented itself when Mayer [1938Th] there encountered a typical case of dissociation during his studies on the microbiology of the 'Tibi grain'. This material, like the 'ginger beer plant' in England, was used in some countries for the preparation of mildly alcoholic, effervescent beverages from sugar solutions flavoured with various fruits. Mayer showed that these grains, which multiply during the fermentation, are composed of a yeast, isolated in pure culture and identified as *Saccharomyces intermedius*, and of a rod-shaped heterofermentative lactic acid bacterium characterized by its ability to produce large capsules of dextran in sucrose media. This bacterium was also isolated in pure culture and named *Betabacterium vermiforme*; it represented the first instance of a rod-shaped, dextran-forming lactic acid bacterium. Mayer had moreover accomplished the synthesis of typical 'Tibi grains' from the pure cultures of the two components.

Starting with fresh 'Tibi grains' from fermenting solutions, Mayer had frequently observed that the lactic acid bacteria colonies developing on sucrose-gelatin plates were all of one kind. But during the isolation of *Betab. vermiforme* it had also been found that different kinds of colonies appeared on glucose-agar plates streaked with suspensions prepared from single, well-isolated colonies of uniform appearance that had grown on solid media. In addition to the more numerous 'rough' colonies, representing the initial type, irregular flat ones were encountered, and it was shown that every suspension of a 'rough' colony invariably gave rise to a certain proportion of colonies of the flat type. It is important to note that this behaviour was observed with cultures on glucose-containing media; here capsule formation never occurs, and this precludes the possibility that contaminating bacteria would be regularly entrapped in the stiff mucus. By the side of the flat, irregular colonies, smooth elevated ones were also found occasionally. Associated with the changes in colonial structure was a loss in ability to produce dextran from sucrose; this applied to both the flat and the smooth types.

A meritoriously critical evaluation of the sequence of events and of many other aspects of the observations finally led to the conviction that the observed 'dissociation' could best be explained as the result of mutations, even though at the time this interpretation was probably the least favoured by microbiologists. The reasoning that led to

this conclusion appears modern even to-day, and it is striking that Mayer carried out some experiments in order to test the possibility that the formation of dextran might be induced in the strains that had lost the capacity to produce capsules in sucrose media, by incubating the latter with killed cells of dextran-producing strains, *i.e.*, by using the approach that several years later gave rise to the spectacular experiments of Avery and associates on the transformation of pneumococci under the influence of desoxyribonucleic acids from different types of these organisms.

That the attitude which yielded the final conclusion was based on a careful appraisal of all the known facts, and was anything but dogmatic is evident from the formulation of the verdict:

'The old idea of Beijerinck's that bacterial variation is the result of gene mutations possesses a degree of probability that is unmatched by any other current interpretation.' (p. 182).

As is well known, this concept came into prominence during the next decade, and it is now universally accepted as the most satisfactory explanation of many forms of microbial variability. The term 'dissociation' has been relegated to limbo, largely because of Braun's [1947] masterly analysis of that phenomenon on the basis of the mutational concept. Braun approached this problem by studying the kinetic aspects of the gradual increase of mutant types in bacterial cultures, which he could attribute to a strong selective influence of the progressively changing medium on the growth of the original organism and of the different variants. In this connexion it is interesting to recall Mayer's observation that cultures of *Betab. vermiforme*, grown in yeast extract-sucrose solutions, show a much lower incidence of mutants than do comparable cultures in glucose solutions or on solid media. This, of course, suggests a similar selective effect, and it accounts for the occurrence of but one colony type of lactic acid bacteria on plates prepared from fresh 'Tibi grains'.

EPILOGUE

In 1939 the 'Delft School', by which here is meant the many associates and students of Albert Jan Kluyver, celebrated the twenty-fifth anniversary of the date on which he had received his Doctor's diploma. On this occasion the Dutch chemical weekly, 'Chemisch Weekblad',

published a special issue containing various contributions in which Kluyver's numerous accomplishments were reviewed. The paper dealing with his eminence as a microbiologist and biochemist ends with the following passage:

'The law will permit him to continue his activities as Professor at the Technological University for nineteen more years. It is to be hoped that he may have the opportunity to devote himself during this time span without interruption to investigations in the area he has chosen as his special field of endeavour, and in which his achievements have already been so numerous. In that event it may assuredly be expected that many more significant contributions from his laboratory will enrich our knowledge, and that many students as well as established investigators will experience the beneficent influence of his personality and institute.' (p. 319).

Unfortunately, this wish has not been fulfilled. There have been long and painful interruptions, and his death, two years before the scheduled date of his retirement, has shortened the expected duration of his association with the famed microbiological institute in Delft still more. In the end it turned out that less than half of the remaining nineteen years were actually available for his scientific pursuits.

Exactly a year after the above-mentioned celebration, Holland was invaded, and for many years suffered under the German occupation. During this period the work in the microbiological laboratory came to a virtual standstill. This explains, for example, why the studies on the methane fermentation, in part already performed prior to the 1939 Helsinki lecture in which Kluyver had mentioned some of the early results, were not completed and published until 1947. And when the war was ended, conditions for the resumption of scientific activities were anything but favourable. Apart from the lack of necessary equipment there were other causes that hampered the development of his programme. In other countries, especially in the U.S.A., scientific investigations had been continued, albeit at a reduced rate and with a pronounced emphasis on application to the war effort. The vast quantity of papers published during this period was not accessible to Kluyver until after the liberation, and when he realized how much had been accomplished, he despaired of ever being able to catch up with the advances made in the interim during which he had been entirely shut off from contacts with science and scientists.

Although physically much weakened as a result of the severe deprivations suffered, and mentally shaken by the horrors experienced during the war years, Kluver set himself the task to assimilate as rapidly as possible the accumulated knowledge, particularly in those fields that most interested him. This led to a number of important reviews, such as those he prepared on the use of isotopically labeled compounds for the study of biochemical reaction mechanisms [1947a, -b]. It also made him aware of the great strides that had been made in the field of enzyme chemistry. Although, as has previously been indicated Kluver had not been overly sanguine concerning the contribution, that could be expected from this approach, the laboratory had acquired, as early as 1938, a Booth-Green mill for the preparation of enzyme extracts from bacteria, and this instrument had been used in attempts to obtain cell-free extracts of luminous bacteria that still possessed the capacity to emit light. Unfortunately, the results had not been encouraging.

Later, Tuynenburg Muys [1949] constructed a simple and effective apparatus for disrupting microbial cells by grinding. But it was not until very recently that la Rivière carried out the first successful experiments with bacterial enzyme preparations in Kluver's institute [1956].

At about the same time a beginning was there made with studies on carbon dioxide assimilation by potentially autotrophic bacteria with the aid of labeled carbon dioxide.

During the early years of his directorship Kluver spent practically all his time in the laboratory, encouraging the students and guiding their efforts by daily discussions in which the status of their work was reviewed in the most minute detail, leading to suggestions for new experiments and approaches. These sessions also served to acquaint the students with pertinent information which Kluver provided from his own vast store of factual knowledge, and to point out potential relationships of experimental results with what at first sight often appeared to be unrelated subjects. In this manner the students gradually became imbued with an increasingly profound appreciation of the true meaning of scientific research, while the close and intensive cooperation between the professor and his pupils led to the development of an enthusiastic and stimulating atmosphere such as is rarely encountered in a scientific laboratory.

But in later years a considerable proportion of Kluyster's time was occupied by numerous official functions, many of which required the preparation of special reports and lectures. Thus, in spite of the fact that he rigorously adhered to his arduous schedule of work, from 9 a.m. till midnight and after, not excluding Sundays, the time he could spend on the supervision and guidance of the work in his institute was severely curtailed. A compensating feature of Kluyster's activities during these later years is, however, that they resulted in the publication of many lectures of great general importance, and one cannot but be grateful for the painstaking efforts and profound thought that went into their preparation. Some of these lectures are reprinted in this volume; they show an increasing preoccupation with the fundamental problems that humanity is facing, and wisdom in the formulation of specific solutions. Thus they are apt to contribute to the development of those attitudes that are so desperately needed in order that we may eventually witness the fulfilment of the ardent wish that Kluyster expressed in the final sentence of the lecture he delivered before the combined sections of the 'Koninklijke Nederlandse Akademie van Wetenschappen' under the title 'Microbe and Life', a lecture to which he was wont to refer as his 'swan song':

'Heavy is the responsibility that rests on mankind;
may we succeed in finding the right way'.

C. B. v. N.

BIOGRAPHICAL MEMORANDA

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References to articles of which Kluyver was author or co-author are found in the 'Bibliography' (p. 527); references marked with Th are included in the list of Doctor's dissertations prepared in Kluyver's laboratory (p. 537), and publications by his students and associates have been assembled in a separate list (p. 540). References to other papers are arranged alphabetically below.

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TWO FUNERAL ORATIONS

OUT of the incomprehensible fullness of his being, Albert Jan Kluyver has given so generously and in so many directions that a multitude of people, here and elsewhere, must have cherished the impression that he belonged to them; and they all must now feel dejected by a deep sense of sadness and loss. He belonged to the world of his science which has bestowed on him its most coveted honours. He belonged to this country which he elevated and to which he brought fame whenever he represented it abroad. He belonged to the city of Delft which he, the world-renowned scientist, loved and served as a good and simple citizen. He belonged to the Delft University, whose brilliant pupil he once had been, and where for so many years he learned, taught, and worked in that unique scientific position that one is tempted to call a 'Regal Chair'. Above all, he belonged to his family, to his children and their mother who preceded him.

If, on the occasion of this last farewell, a few words are spoken on behalf of the entire university, this is done in clear recognition of the fact that within its confines, too, his activities and solicitude were so diverse that many will experience the desire to bear witness to their esteem and sorrow. In the first place we think of those who were his collaborators in the secluded community of the quarters that were his home and his workshop; who surrounded him with their daily cares; and on whom the thought that they will never see him again must leave an impression of unutterable emptiness. We think of the academic youth, the students to whom he was so deeply devoted; to whom his anxiety and vigilant compassion went out during the dark years; and whom, only a few days ago, he addressed on the science of invisible life. I may speak, too, in the name of the Board of Trustees and the Department of Chemical Technology who but recently, impelled by their admiring respect, had jointly decided upon exceptional provisions for the chair of microbiology, just because it was Kluyver's. And I also speak on behalf of the Academic Senate, simply to say that from its midst has been taken he who was incontestably the foremost.

In our memory he stands in his accustomed place in the council chamber, holding forth in his characteristic attitude, advising and warning and offering a solution; and we knew that it was right to accept his council and to pursue the path that he laid out. On special occasions we would ask Kluyver to be our leader, and we felt secure and protected. To him we could go with our troubles, and we were met with endless patience for our questions, and received attention and understanding, consolation and strength.

Truly, towards him whom we have lost, all words seem powerless. His profundity and versatility silence us. But we have always known that he was not just an exceptional scientist, but first and foremost a great and complete human being, and that his talents were alloyed with a noble steadfastness of character, and a tender, deep-seated sensitivity. About inessentials he could talk playfully, even flippantly; but he never compromised when faced with fundamentals. In him there was room for the significant side by side with the fleeting, and without endangering the unity. He who worked at the farthest borders was at the same time attentive to the daily chores.

And another combination of opposites: his mind that was so supple, full of esprit, and open to the unknown, liked to preserve an old but meaningful form. In his style of life the dignity of the magistrate could harmoniously be blended with the utmost simplicity. Order, justice, and balance conferred upon him the invulnerability on which one could build and trust. The word 'classical' comes to mind; and another, 'self-possession' in its multiple connotations.

This rich life has now come to an end. In a spirit of reverence and affection, deeply moved and in sorrow, the University commemorates him, and is grateful for what it has been vouchsafed in the person of Kluyver.

O. Bottema

ON this occasion I wish to speak in the capacity of a friend – I may even say an intimate friend – of the deceased. We were bound by a friendship that, begun in September, 1905, when we first registered as students in Delft, has now come to an end.

When, after having spent some years in the tropics, Kluyver returned to Delft to occupy Beijerinck's chair in 1921, that friendship continued without interruption. Both of us were married; both of us reared five children who also became mutual friends. Never has there been a single note of discord; small wonder, then, that the friendship was firm, and considered as something self-evident.

Thus I have been in a position to observe Kluyver throughout the 35 years during which he developed into an ornament of the Technological University and a scientist of international repute. It is the man Kluyver whom I want to bring back to your memory in these moments.

It is amply recognized that Kluyver gradually built up a school of microbiologists. What is a school? It implies that a group belonging to a younger generation conduct experiments and publish their results under the guidance of a mentor, and that they all bear the imprint of some fundamental idea of the master.

How could the master do this? Firstly because his fertile mind could initiate profound concepts; but also because he cooperated with his assistants and students; because he knew how to inspire them with ardour for their science.

This, only a great man can do. One will never see a school develop if the teacher does not possess great human qualities. Kluyver has lived and toiled for his students. That was particularly evident when they took their degree under him and were writing their theses. It often happened that, despite pressure from his family, Kluyver did not take – or postponed taking – a vacation because he stayed behind to work with the pupil; occasionally he might take the student along. And then Kluyver would concentrate on that work with his pupil for entire weekends, and not infrequently have his pupils stay on as guests.

And in this manner much was accomplished. Every publication was permeated with the fundamental concepts of the master. And every pupil departed with gratitude towards his teacher; every one had gained much, not only in scientific knowledge, but also – some more, others less – worldly wisdom which they could carry as valuable lug-

gage for life; for Kluyver was not only a very gifted man with an exceptional intelligence and insatiable curiosity, he was also a wise man.

What is wisdom? 'Wisdom is a native gift of intuition, ripened and given application by experience, for understanding the nature of things, certainly of living things, most certainly of the human heart'. These are the words of T. S. Eliot, spoken when he received the Hanseatic Goethe prize for 1954. But I can also illustrate, by some eminently practical examples, what Kluyver's wisdom implied.

He never rebuked; he never told any one off, as so many of us are apt to do. He never became irate; during those fifty years I have never seen him angry. He was a wise man.

This he was also to his students and pupils. His wisdom towards them included also the recognition that not all persons are alike, that not every one was as he; and he not merely tolerated, but actually encouraged different personalities. There was but one human trait he disliked: pretentiousness, would-be learning, hypocrisy, insincerity.

Is it surprising that he was venerated by his pupils, and that, when he had been professor for 25 years and declined a grand celebration, his pupils gave him a dinner party at which they toasted him so profusely and so touchingly?

Kluyver was also a man of style. Has not Buffon said that 'Car le style, c'est de l'homme même'? This was a most essential element of his character. He was a man of decorum, and he despised vulgarity.

His feeling for style was also expressed in his brilliant lectures on his specialty and related matters. Yet one should not think that it came to him easily, by some sort of intuition. It was achieved through hard work, by revising, by pondering every single word.

In his conversation, too, he was original and singular. His diction was intensely personal, sometimes spiced with expressions that had their origin in the district where his father and mother had been born, and where his grandfather had owned a mill.

He liked slogans, which he replaced by new ones every few years. During the 'thirties, when he was exceptionally busy, I remember that the favorite one was: 'This is a great and terrible world'. But in his later years he had become more resigned, and was wont to say: 'Well, that's how it is'.

His conversation was brilliant in another respect. In a few words he could outline a situation, either in science or in politics. He often

explained to me some microbiological problem, fully clarifying the subject in a few terse sentences.

In spite of the fact that many honours were conferred upon him – honorary doctorates, the Hansen- and Copley medals, election to many foreign scientific societies – Kluyver remained a modest man. He never prided himself on these distinctions; he always made it appear as if he owed them to some accident or chance. When he had been awarded the Emil Christian Hansen medal in Copenhagen and had returned to Delft, his students and associates celebrated the occasion by presenting him with an enormous cake bearing the inscription, ‘I am only a simple man’. It was one of his own, characteristic slogans.

Kluyver seldom spoke of things to come; rarely mentioned the fact that, at the end of 1956, he would have to give up his beloved home, and a year later his old laboratory. It was as if he himself doubted that he would witness these events.

And now this beautiful life has suddenly been cut off. Former pupils, spread out over the entire world, will share these moments with us. They will gratefully remember their master. Every one of them has taken along something he had received from him; in difficult moments they will all be inclined to ask: ‘What would Kluyver have said?’

Nothing is ever lost in this world. Kluyver is no more; but the seed he has sown on the fields of science and in our hearts has long since germinated, and those who have known him well will retain the memory of an eminent scientist, but above all of a complete human being with a big heart.

And if we can do this, how much more strongly will these memories remain alive in his children who have known him in other ways, and who will be grateful that they had so excellent a father.

The relation with his children I cannot better express than in the words in which he characterized his own education, and which are taken from his inaugural lecture in which he addressed his parents thus: ‘The education you gave me was characterized by acts of love rather than by verbose theory’.

This friend of half a century, who actually never knew any rest, – may he now rest in peace.

A. v. Rossem

PART TWO

SELECTED PAPERS

MICROBIOLOGY AND INDUSTRY

'This enables the chemist to regard micro-organisms as co-practitioners of his craft, and the chemical achievements of these humble agents have continued to excite his admiration since they were revealed by Pasteur.'

('The Laboratory of the Living Organism', Presidential Address delivered by Dr. M. O. Forster, F. R. S. to Section B of the British Association for the Advancement of Science, at Edinburgh on September 1, 1921.)

INSTRUCTION at a present-day Technological University has gradually expanded into a grand symphony of pure and applied science. Only the exceptionally practiced ear can adequately value the rôle of the individual instruments, *i.e.*, the various branches of these sciences, separately. Besides, scientists will but rarely be provided with an opportunity to perform as soloists before an audience that is not composed exclusively of professional experts. The newly appointed professor who, in accordance with tradition, embarks on his career with a public address faces a task that calls for an appraisal of two aspects. This is so because on the one hand the listener may be inclined to attach to this address a special significance in that it will permit him to evaluate the speaker's ideas concerning the manner in which the latter intends to carry out the duties of his new office. On the other hand, the introductory remark implies that this lecture is also important from the point of view of his specialty because it cannot fail to influence the position that will be assigned to his branch in the composite. The latter consideration weighs more heavily on me than personal responsibility now that, after a lapse of nearly a quarter century, a spokesman for microbiology may once again request the attention of the assembled representatives of technological higher education. And this is all the more so because of the fact that it is my privilege to speak in the same city where Antony van Leeuwenhoek, the father of microbiology, used to live and work.

As a subdivision of biology, microbiology is perhaps in greater need of an occasional opportunity to make itself heard than any other subject taught at this university. The reason is that at first sight there

appears to be a chasm between the science of life and modern engineering sciences that precludes any possibility of co-ordination. Nevertheless, it is my firm conviction that a closer consideration will reveal the situation to be otherwise, and that, side by side with the other fields of study, microbiology may lay claim to a profound interest on the part of all those who are concerned about the education of the prospective chemical engineer. Hence my aim to-day will be to make you partisans of this view, and for this purpose I shall discuss the rôle that microbes play in human economy.

Human society depends nowadays, among many other things, on a large number of organic substances for its multifarious needs. A gigantic organic-chemical industry, this term to be understood in its broadest sense, performs the requisite conversions of organic compounds. First of all I want to examine the question whether in the present day and age microbes may still fulfil a function in this industrial transformation process.

Obviously, there is every reason for posing this question. For, despite the fact that in the beginning of the previous century the synthesis of the manifold organic substances was considered to be the exclusive prerogative of living organisms, there has gradually developed a strong reaction against this notion.

A professor of organic chemistry is not likely to pass up the opportunity to refer in his inaugural address to the discovery that was made on February 22, 1828, and to do so with consummate satisfaction. And rightly so; for on that memorable day the barriers which until then had sharply divided the products of animate from those of inanimate matter were shattered in consequence of the accomplishment of the celebrated chemist, Friedrich Wöhler, who had succeeded in converting ammonium cyanate into urea. An endless field for study, inaccessible till that moment, was thereby opened up for the chemist. And I need not belabour the point that chemistry and chemical industry have nobly acquitted themselves in this respect, and scored prodigious successes. The number of organic substances that the chemist has learned to prepare in his laboratory runs into the hundreds of thousands, and the application of these discoveries has caused sweeping changes in the world's enterprises.

For the time being the organic-chemical industry still remained dependent for its raw materials on the fossil and extant vegetable kingdom. The conversion of carbon dioxide of the atmosphere into organic matter, accomplished in the leaves of green plants under the influence of solar energy, remains a problem that the chemical engineer has so far failed to solve, at least in an economically practicable manner.

Now it is in particular in connexion with the utilisation of the fossil raw materials, coal, bitumen, lignite, and petroleum, that the organic-chemical industry can boast of its greatest triumphs. Even though in many places the sugar-, oil-, and starch industries have been enormously expanded, one should not lose sight of the fact that in all these cases the plants, *e.g.* the sugar cane, coconut, or potato, are the true manufacturers of the desired raw materials; it is merely the isolation therefrom of the ingredients most valuable to man that is carried out in the factories.

The world's reservoirs of fossil raw materials are, however, far from inexhaustible; on the contrary, sundry investigations concur in leading to the conclusion that several of these products will be depleted in the not too distant future. Their ever increasing exploitation makes one fear for the worst in this respect. In a study of the energy provision of the U.S.A. Steinmetz has calculated that since 1870 the coal production has increased on the average by 6.35 per cent annually. From 10 million tons in 1852 it has risen to 100 million tons in 1882; in 1920 it has probably exceeded 1000 million tons; and, if the increase continues at the same rate, it should amount to 10,000 million tons by 1958. Similar considerations generally apply to other countries, except for the fact that the world war has caused temporary perturbations. Although Svante Arrhenius recently estimated that the known coal deposits in the world would be sufficient for 1500 years on the basis of current consumption data, the Steinmetz figures indicate that the time at which coal rationing will have to be imposed in certain parts of the world is apt to be reached considerably earlier, in fact, in the foreseeable future.

The prospects for the petroleum industry are even more dire. Authorities whose judgement carries weight do not consider it improbable that within a few decades the maximum rate of petroleum production will be reached. Equally pessimistic views have frequently

been expressed in connexion with the problem of the world's energy provision. Nevertheless, there is always the bright prospect that before a state of emergency will have arisen, human cunning will have succeeded in fixing solar energy directly, *i.e.*, without the aid of the plant world, in a form that is useful to society. Even the possibility that some day man will be in a position to exploit the enormous intra-atomic energy cannot any longer be relegated merely to the realm of fiction, as indicated by scientists of the stature of Richardson and Rutherford.

But the exhaustion of fossil fuels is important not only from the point of view of energetics. I have already mentioned that they supply a significant fraction of the raw materials needed by the organic-chemical industry. The gradual depletion and the resulting higher price of the fossil raw materials will consequently entail that the chemical industry will gravitate more and more towards procuring its starting materials immediately from the present-day plant world.*

Thus the intensification of agriculture, and its development along lines of modern industry, will in the future become more and more the order of the day. This will mean that the chemical industry will be provided primarily with a few staple agricultural products whose value derives largely from their content of the three major groups of substances, the carbohydrates, oils, and proteins. And then it are particularly the carbohydrates – the sugars, starches, and cell-wall constituents – that will become prominent as raw materials.

Now the specific advantages of microbiological mechanisms are strikingly apparent in the transformations of carbohydrates into numerous other products. With an ease that elicits the organic chemist's envy various microbes can split the glucose molecule, each one yielding its own more or less specific products. Thus the common yeast, *Saccharomyces cerevisiae*, furnishes alcohol and carbon dioxide; *Lactobacillus fermentum* and related types produce significant quantities of lactic acid; Fernbach's *Bacillus macerans* manufactures butanol and

* The fact that we can currently observe diversions in the opposite direction, for example in the manufacture of alcohol from coal *via* calcium carbide, acetylene, and acetaldehyde, or in the production of synthetic fats from paraffins by way of their oxidation to fatty acids, does not detract from the validity of the general argument. By increasing the utilization of the fossil raw materials such developments can only hasten their depletion.

acetone; the recently isolated *Bacillus acetoethylicus* yields acetone and ethanol; Beijerinck's *Granulobacter saccharobutyricum* makes butyric acid; Duclaux's *Tyrophthrix tenuis*, dihydroxyacetone. Various moulds of the genus *Citromyces* may give rise to the production of appreciable quantities of citric acid from glucose; Wehmer's *Aspergillus fumaricus* converts it into fumaric acid with very good yields. It would not be difficult to augment these examples with many others, but I fear that this would become tiresome.

The above survey may, however, suffice to make you realise that a substance such as glucose, easily procurable by purely chemical or biochemical methods from a staple product such as starch, can be converted into diverse products with the aid of micro-organisms. And it seems to me that these are exactly the compounds that are of prime importance to the organic chemical industry as building blocks for various syntheses. I believe that no organic chemist is likely to contradict me when I say that the purely chemical manufacture of the above mentioned substances from glucose will cause him far greater difficulties than one is apt to encounter in the corresponding biochemical transformations.

In his opening address of the Congrès de la chimie industrielle, 'L'avenir de la chimie organique', none other than Sir William Pope has quite recently emphasized the considerable difference between organic chemistry, both of the past and present, and the conversions that proceed in living organisms. On the one hand Pope notes the high temperatures and the powerful, though rather unspecific chemical reagents that are still indispensable to the chemist; on the other hand the extremely selective mechanisms of the living cell, functioning at ordinary temperatures. It is true that Pope points out that the methodology of modern organic chemistry shows an increasing tendency to approximate that of biochemistry, since the application of catalysts, and the consequent use of colloidal reaction media, are practiced more and more by the organic chemist. But this does not alter the fact that the distance that will have to be traversed before the organic chemist possesses a set of catalysts that would enable him to perform the conversions summarized above without the aid of microbes still appears very great.

This raises the question whether biochemical conversions, proceeding under the influence of micro-organisms, are currently of importance for the organic-chemical industry.

Let us first of all consider the alcoholic fermentation of sugars. The enormous economic significance of biochemical alcohol manufacture, so universally practiced by primitive and by the most highly civilized societies alike, does not need extensive illustration. Simmonds estimates the average annual production by the seven most important producing countries over the period 1909-1913 as more than 436 million Imperial gallons of 100 per cent alcohol. If one realizes that the practice of single-cell culture methods has convincingly shown that potentially one single yeast cell, of which around 2.10^{10} make up one gram of compressed yeast, suffices for the production of so vast a quantity of alcohol, this will provide a striking demonstration of the *macro*-achievement of a *micro*-organism.

The above-mentioned estimate of 436 million gallons includes, however, 310 million gallons that have served for human consumption. One might therefore be inclined to think that an extension of prohibition over the rest of the world, following the example set by the U.S.A., might cause irreparable damage to this industry. But if such an extension were to be introduced gradually, such consequences need not be feared on account of the general situation that I have already indicated, *viz.*, that the potential exhaustion of fossil fuels cries out for substitutes derived from the present-day plant kingdom. As mentioned earlier, one cannot sustain high expectations with respect to future petroleum production. In even stronger measure does this apply to the production of petrol; as is known, this occurs in reasonable amounts in some crudes only. In view of the undreamt-of expansion of automobile traffic it will be evident that serious concern is felt in various circles in connexion with the possibility that the petrol production might not keep pace with the increase of motor vehicle traffic. For a considerable time a search for petrol substitutes has already been instituted, and universally the conviction has been gained that industrial alcohol offers the best prospects. In 1918 the British Government therefore appointed an 'Alcohol Motor Fuel Committee' that was charged with making a study of an eventual large increase of the alcohol industry, and at the same time of the difficulties inherent in the use of alcohol in petrol combustion engines. Aided by national committees in different parts of the Empire, this committee has issued a report in which it calls attention to the particular suitability of the tropical and subtropical regions for an increased carbohydrate pro-

duction with conjunct manufacture of alcohol. The drawbacks initially attaching to the use of alcohol in petrol combustion engines appear to have been conquered satisfactorily both in South Africa and in Australia. Hence the future of the biochemical alcohol manufacture seems to be assured.

While discussing this industry I cannot forego mentioning specifically the successful application, in France and a few other countries, of the 'amylo-process', because this supplies one of the outstanding examples of the imaginative use of micro-organisms as aids in chemical technology. It also demonstrates that the primitive procedures of Oriental peoples often contain the germ of a methodology that can effectively be used in competition with Western processes.

It has long been known that from olden times the Chinese have possessed the secret of preparing alcoholic beverages from rice. For this purpose they use a material that has commonly been designated by European investigators as 'Chinese yeast', and that was first studied in 1885 by Calmette in Indo-China.* According to a formula obtained by Calmette, the preparation of 'Chinese yeast' requires no less than 46 ingredients, most of which are parts of plants with special flavouring qualities. The active principle, however, consists of the microbes that are always present in these ingredients, and among which a mould, probably belonging to the genus *Mucor*, is particularly predominant. This mould has been obtained in pure culture; it is characterized by a very strong diastatic power, *i.e.*, the ability to convert starch to sugars.

Calmette then attempted to use the diastatic properties of this and of related moulds also in Western European alcohol industry, for here, too, starch-rich raw materials are converted into sugar by diastatic enzymes, as, for example, in breweries, where one generally depends on the diastase found in malt, *i.e.*, germinated barley. Now the application of mould diastases seems to offer a number of advantages, such as the fact that the hydrolysis of starch yields virtually no non-fermentable dextrins. After many reverses Calmette's collaborators have finally succeeded in sufficiently eliminating the practical difficulties, so that a few years ago Delamar estimated that 6 million hectolitres of alcohol were produced by the amylo-process. In due course the initial-

* A very similar product is generally available in the Dutch East Indies under the name 'ragi'; it was investigated by Went and Prinsen Geerligs.

ly employed moulds have been replaced by others that are even more satisfactory; and currently only those known as *Rhizopus* Delamar and *Mucor* Boulard find application.

Still dissatisfied with the results, Effront and Boidin have aimed for still higher goals. They had found that in both the malt- and the amylo-process powerful proteases are operative in addition to the amylases. This causes the major part of the proteins present in the raw material to be converted into simple, water-soluble nitrogen compounds that are lost in the course of further operations. Moreover, even the use of mould diastase requires a rather costly pretreatment of the starch-rich material with steam under pressure. These investigators therefore attempted to prepare a potent diastase that can act on native starch, and that leaves the proteins largely intact. In this manner the nitrogenous material can be recovered after the alcohol has been distilled off, and used as fodder. They ultimately succeeded in finding the desired biochemical catalyst in a specially adapted culture of *Bacillus mesentericus*. If properly prepared, one unit weight of such a culture is capable of hydrolysing 1,000 unit weights of starch. Following the treatment with *Bac. mesentericus* the raw material is subjected to a brief action by *Rh.* Delamar in order to produce exactly the minimum amount of soluble nitrogen compounds needed for a good development of the yeast during the third stage of the process. Thus one sees how in Effront's modification of the amylo-process the entire conversion of starch into alcohol is accomplished by the successive operations of three different micro-organisms.

Proceeding now to a consideration of some other biochemical conversions, I may state that for the technical manufacture of lactic acid the fermentation method possesses a virtually undisputed monopoly. Chapman estimates that in 1909 Germany produced no less than 1500 tons of lactic acid for export, over and above the amount used inside the country. Also in Holland the commercial manufacture of lactic acid has lately been introduced.

Most intriguing is furthermore the history of the biochemical manufacture of acetone. During the years of the great rubber boom chemists in many countries tried to find a method for the manufacture of synthetic rubber. The British chemist, Perkin, devised a procedure in which butanol was used as the starting material. In conjunction with this process the French bacteriologist, Fernbach, developed a method

for the production of butanol and acetone from potato starch by fermentation with a bacterium he isolated, and which is probably identical with *Bac. macerans* studied in 1905 by Schardinger. On account of the gradual changes in the rubber market the manufacture of synthetic rubber never got started. But in 1915, when a great demand for acetone developed in England for the manufacture of explosives ('cordite'), Fernbach's pupil, Weizmann, succeeded in adapting Fernbach's procedure to the large-scale production of acetone.* From England this process was exported to Canada where the 'British Acetones Ltd.' built a large factory in Toronto for the new process. In 1918 this factory contained no less than 22 functioning fermenters, each one with a capacity of 30,000 gallons. From Canada the process was introduced into the U.S.A.; meanwhile, Fernbach had succeeded in developing his process on a technical scale in France.

During peace-time the commercial success of this process obviously depends on the possibility of finding a ready market for the butanol which is produced in an amount twice as great as that of the acetone. Butanol had not previously been used in chemical industry; various possibilities for a potential utilisation of this substance have already been suggested, but as yet it is not possible to express a final verdict concerning their feasibility.

Meanwhile Northrop, Ashe and Morgan had isolated their *Bac. acetothylicum* which produces acetone accompanied by ethanol instead of butanol. Furthermore, subsequent investigations showed that this bacterium is not particular as far as its nutrition is concerned; it produces the same substances from pentoses in quite satisfactory yields. This opens up a means for economically disposing of the corn cobs, now representing a waste product which, in the U.S.A., amounts to 20 million tons annually.

Whatever else may develop from these industries, it may suffice to state that estimates show that, at the time of the armistice, 5,000 tons of acetone and twice that amount of butanol had been produced in the U.S.A. alone, and this owing to the activity of a microbe that twenty years ago had probably never been considered worthy of human attention. In this connexion I may point out that the thousands

* Whether Weizmann used the initial isolate of Fernbach is not quite certain. In one of his later patents he recommends the use of *Granulobacter pectinovorum*, discovered by Beijerinck and van Delden as a causative agent of flax retting.

of cultures employed in these industrial fermentations may well have originated from one bacterial cell whose dimensions, in comparison with those of the earlier mentioned yeast cell, are very small indeed. If we further realize that in a relatively short time that single, minute cell has given rise to the construction of many imposing factories in various parts of the world, then it is unavoidable not to be impressed by the enormous powers that reside in even the smallest living entities. Then, too, we cannot escape the notion that, in view of the thousands of microbes that so far have only superficially been investigated, and of the unknown numbers of microbes that occur on earth but have never yet been observed by man, a rational exploration and exploitation of the powers of the microbial world may still bring many beneficial results to mankind.

This possibility is strikingly illustrated by some procedures worked out in Germany during the war emergency. I refer to the microbiological production of fat and protein. It had long been known that under certain conditions various micro-organisms accumulate fats and oils as intracellular reserve products. Encouraged by the great demand for fat during the war, Lindner made a careful study of this phenomenon, hoping thereby to accomplish a technically feasible procedure for the conversion of carbohydrates into fats. In a sample of some material collected from the sap flow of a birch tree and which his pupil Schrettenfeger had sent Lindner from the Eastern front, the latter discovered an organism, *Endomyces vernalis*, that appeared eminently suitable for such a fat synthesis. It was found possible to obtain good yields of this organism with a fat content of 60 per cent, calculated on a dry-weight basis, by growing it in sugar media with a small amount of ammonium sulphate as nitrogen source.

A comparable situation is encountered in the microbiological protein synthesis, also evolved in the 'Institut für Gärungsgewerbe' in Berlin. Starting from the consideration that, after elimination of the bitter ingredients introduced by the hops, the brewery yeast represents an eminently suitable, protein-rich fodder, Lindner further investigated the possibility of manufacturing a protein-rich yeast product from readily available inorganic nitrogen sources, primarily ammonium sulphate, reasoning that, at least during periods of severe food scarcity, such a process might be economically important. As a result of this investigation many factories in Germany actually pro-

duced a 'mineral yeast' during the war, at which time it acquired importance as food for both man and animal. For this purpose a yeast was used that is closely related to the common alcohol yeast but that, when cultivated in shallow layers of liquid exposed to air, displays virtually no fermentative activity so that under these conditions a strictly aerobic respiration is accompanied by a copious multiplication. On Chapman's [1921] authority, who spoke in this connexion of 'true Teutonic enthousiasm', I may state that it was this process that tempted Hayduck to the pronouncement that not until man is in a position to convert his evening newspaper so rapidly into sugar that the protein produced therefrom can be consumed the next morning at breakfast will one of the greatest problems of this century have been solved!

It is also worth mentioning that the reverse of the last-mentioned process, *viz.*, a microbiological conversion of the organic nitrogen compounds present in waste materials into inorganic nitrogen compounds is the basis of a native British Indian industry that is currently still most important in that country. It may surprise many of you to learn that before the war British India, despite a far from negligible native consumption, exported on the average 15,000 tons of salpetre annually, while during the war this figure reached approximately 50,000 tons. All of this nitrate had been produced by the natives in a primitive manner, by making use of the powerful bacterial nitrification process that, in the tropics, can proceed under very favourable conditions.

The preceding remarks have opened up many unsuspected prospects for applied microbiology; nevertheless, the existing possibilities have by no means been exhausted. Thus far I have discussed micro-organisms as biochemical catalysts more or less on a par with the catalysts of pure chemistry. But this implies a serious undervaluation that calls for a redress. Another aspect is evidently revealed by the power of organisms to reproduce, which consequently means that, in contrast to what happens to purely chemical catalysts, an enhanced production of biocatalysts occurs during microbial conversions as a result of cell multiplication. In addition I must emphasize the great adaptiveness that appears to be inherent in living organisms. In this connexion I think of the surprising modifications in the metabolic properties of a cell under the influence of chemical changes in the medium in which

it lives. Striking examples of this sort have become known, especially during recent years, and they do not suffer from a lack of technological significance.

A first instance may be derived from an investigation of the American microbiologist, Currie. Whereas oxalic acid, in addition to carbon dioxide, had been found as a significant by-product of the carbohydrate metabolism of the intensively studied mould, *Aspergillus niger*, Currie succeeded in demonstrating that the oxidation of carbohydrates by this mould proceeds in consecutive stages, and that citric acid is one of the earliest intermediate products. Currie further proved that, by an appropriate choice of environmental conditions, the metabolism of *Asp. niger* can be so guided that citric acid production is enhanced, while the further oxidation of this product to oxalic acid and carbon dioxide is inhibited. Thus he was able to increase the citric acid production from sugar to 50 per cent, whereas under the conditions commonly employed never more than trace amounts of this substance are found.

Here is another example. Pasteur already knew that during a normal alcoholic fermentation a small quantity of glycerol, ordinarily about 2–3 per cent of the fermented sugar, is formed in addition to the major products, alcohol and carbon dioxide. When during the war Germany suffered from a serious shortage of glycerol, owing to the deficit of fats, various investigators raised the question whether it might not be possible under special conditions to induce the yeast to an increased glycerol production. Probably fully independently, this problem was simultaneously solved by Connstein and Lüdecke, and by Neuberg and collaborators in Germany, by Schweizer in Switzerland, and, later, by Eoff and co-workers in the U.S.A. All four groups found that the yield of glycerol can be increased to 25–30 per cent of the sugar by adding appreciable amounts of sodium sulphite to the normal, sugar-containing media. In Germany this process has been used on a large scale.

What makes this discovery particularly interesting is the fact that Neuberg observed that besides glycerol only very little alcohol was formed, while a large amount of acetaldehyde, equivalent to that of glycerol, was recovered. I cannot here enter into a discussion of the enormous significance that this discovery has exerted on our interpretation of the chemical conversions that take place during a normal

alcoholic fermentation. I shall only emphasize that a microbe, used as long as man remembers for the manufacture of alcoholic liquors, and unquestionably studied more thoroughly than any other microbe, after half a century of intensive microbiological research suddenly appeared capable of performing hitherto unimagined chemical conversions. If such thoroughly studied organisms as the ubiquitous yeast and *Asp. niger* can occasion such surprises even to-day, the thousands and thousands of microbes, thus far studied only cursorily if at all, may yet offer limitless possibilities for biochemical transformations that may also be important from an industrial point of view. But at the same time we realize that the inflexible concept of microbes as catalysts capable of performing but one specific conversion implies a serious underestimate of the capacities of these organisms, and we must bow before the impressive potentialities of life.

Now one might justifiably object that, from a commercial point of view, many of the above-mentioned processes have not been a lasting success, and have not or only barely been able to weather the economic post-war competition. To this I can only reply that I have chosen these examples because they seemed to involve some ideas of general significance. It should, however, not be concluded that they represent the only examples of an industrial application of micro-organisms. Had I wished to restrict myself to long established and renowned industries in which microbes play a leading rôle, I might have discussed the manufacture of wine, beer, and vinegar; the dairy industry; the retting of fibres; the production of drinking water; the practice of sewage disposal; etc. I might then also have dwelt on a few flourishing primitive industries of the Orient, such as the 'ontjom'- and 'tempé'-industry in Java, an industry that may well await its Calmette in order to gain significance for the tropical oil industry which is now conducted on a Western pattern.*

But I might rightfully be convicted of pronounced one-sidedness if I tried to advocate the misapprehension that at all times microbes are the benefactors of the industrialist. Far from it; there are numerous cases in which the microbes interfere in a most undesirable manner in the production process.

* Owing to Went's investigations much has been learned about the microbiological process of 'ontjom' and 'tempé' production; nevertheless, a rational industrial application of these findings has not as yet been attempted.

It is well known that the oleomargarine industry, which during the war years underwent a rapid expansion especially in England, was seriously threatened by the poor keeping qualities of its product. Oleomargarine appeared to be particularly vulnerable to the deterioration known as rancidity. Owing to the outstanding studies of Jacobsen we now know that rancidity, both of vegetable fats and, as Orla-Jensen had proved, of dairy butter, is caused by micro-organisms. It will therefore be necessary to attach great value to various means by which the product can be protected against contamination during the successive stages in its production. If we may believe a recent study of Stokoe it will even be necessary to pay the closest possible attention to such measures already during the production of the plant oils that are used as the starting material in the oleomargarine industry.

During the war large quantities of sugar, produced in tropical countries, could not immediately be shipped, and thus had to be temporarily stored there. In this case, too, deterioration was soon observed. Kopeloff and collaborators in the U.S.A., and Amons in Java, could unequivocally demonstrate that the deterioration was again the result of microbial activities. In some cases the source of the contamination could be located in the factories, and measures could thus be devised to restrict the deleterious effects to a minimum. Browne estimates that these microbes caused the Cuban sugar harvest of 1916 alone to suffer a loss in value of \$ 1,500,000.

Let me finally remind you of the industries concerned with the preservation of foodstuffs, which owe their existence to the ubiquitousness of spoilage-provoking microbes. Guided by intelligent pure food laws, the large American canning industries have increasingly been forced to adopt practices that are based on the results of scientific investigations dealing with the destruction of micro-organisms as a function of particular conditions. This certainly is an example worthy of being emulated!

Whereas microbiology thus serves industry firstly by providing leads for the proper management of biochemical transformations, and secondly by developing adequate techniques for a rational elimination of unwanted micro-organisms, there is yet a third area in which it can unfold its powers to the benefit of industry. In this connexion I have in mind the indispensability of scientific investigations for the proper

management of every industry. I may here refer, for example, to the studies of Pfeiffer showing that the rate at which various kinds of wood are decomposed under the influence of methane producing bacteria may provide important indications as to the durability of these materials in actual practice.

Biochemical sugar determinations can certainly be of importance in the scientific control of the fermentation industries, in particular if new raw materials are being used; a good example is furnished by the gradually developing alcohol manufacture from sawdust.

And microbiological studies can be of importance even for the more remote petroleum industry. With the aid of micro-organisms that can decompose the hydrocarbons of the paraffin series, and that had previously been studied by Söhngen, Tausz and Peter recently accomplished the isolation of naphthenes, not attacked by these bacteria, from mixtures of naphthenes with paraffin hydrocarbons. This represents a separation that offered well-nigh insurmountable difficulties when attempted by purely chemical methods, even in Engler's laboratory.

It would not be difficult to cite many more instances, but I must already have taxed your attention unduly.

The famous biologist, Huxley, calculated at one time that the economic advantages that France reaped from Pasteur's fundamental microbiological discoveries were sufficient to pay, during a period of only twenty years, for the entire war debts incurred in the war of 1870—1871, debts that were then considered stupendous. Is it too bold to propose that, as a consequence of the above discussion, we may expect that during the next few decennia microbiological science may significantly contribute to an alleviation of the economic consequences of the world war?

Before closing I must, however, dispose of a misapprehension that may have arisen out of the previous remarks. I have used the time at my disposal largely to give you an idea of the great importance of microbiological applications in industry. But I should not like to leave you with the impression that the study of general or theoretical microbiology, which I shall also have to teach, appeals less strongly to me; nothing could be farther from the truth.

When Leibniz once urged Van Leeuwenhoek to see to it that his method for grinding microscope lenses would be transmitted to later generations, Van Leeuwenhoek replied:

'To train young people to grind lenses, and to found a sort of school for this purpose, I can't see there'd be much use: because many students at Leyden have already been fired by my discoveries and my lens-grinding, and three lens-grinders have gone there in consequence; to whom the students have repaired, to learn how to grind lenses. But what's come of it? Nothing, as far as I know: because most students go there to make money out of science, or to get a reputation in the learned world. But in lens-grinding, and discovering things hidden from our sight, these count for nought. And I'm satisfied too that not one man in a thousand is capable of such study, because it needs much time, and spending much money; and you must always keep on thinking about these things, if you are to get any results. And over and above all, most men are not curious to know: nay, some even make no bones about saying: What does it matter whether we know this or not?' *

Even though, after a lapse of 250 years, our opinions as to the economic importance of the microbial world may differ from those held by Van Leeuwenhoek, I should not wish to be counted among those to whom knowledge is important only if in a material sense one 'can get something out of it'. I, too, am not insensitive to the fascination which the study of nature and the struggle to penetrate into its tenaciously guarded secrets holds for the investigator. Moreover, I keenly realize that the study of general microbiology is a primary prerequisite also for future progress in the realm of microbiological applications, and this at the same time justifies the inclusion of this purely scientific subject in the curriculum of a technological university.

In a noteworthy paper Rahn [1921] has recently made a plea for theoretical bacteriology which he characterizes as the foster-child among the sciences. He points out that, in contradistinction to most of the latter, in Germany bacteriology has been able to develop only as an accessory, as the handmaiden principally of medicine and agricultural science, from the very start. Not a single German university can boast of the inclusion of an institute for theoretical bacteriology; nowhere does bacteriology find a refuge where, in peace and quiet, it may be studied for its own sake.

It is true that bacteriology, as a subdivision of botany, can right-

* *Ed. note:* This passage has been copied from Clifford Dobell's [1932] superb translation of Van Leeuwenhoek's reply (p. 325).

fully engage the interest of those who hold positions in the universities' botany schools, and several amongst these botanists have made meritorious contributions to microbiological science. Nevertheless, if we survey the extent of the plant kingdom, and recognise how relatively modest a place the microbes occupy therein, we realize that the professor of botany can and should devote no more than a small fraction of his time and efforts to bacteriology. But the present status of bacteriological science imperatively calls for a large number of investigators who can dedicate their entire life to studies in this field.

I shall mention but a single example in support of this claim. It is well known that even to-day there exists an exasperating confusion in the area of the systematics of the *Schizomycetes*. In this respect the students of bacteriology have even been compared to insulars who, each one sitting tight on his own little island, are wont to hurl minor verities at each other across oceans of misunderstanding. Surely this picture is hardly painted too darkly!

In consequence of the minute dimensions of the bacteria, their morphological characteristics cannot have the same significance in bacterial systematics as they do in the taxonomy of the higher plants. Hence physiological and ecological properties must occupy a far more prominent place in bacterial taxonomy, and, together with morphological criteria, they must be wielded into an encompassing and harmonious whole. Thus it follows that an intensive investigation of the multifarious bacteria, extended in many different directions, is required to escape from the present impasse.

Meanwhile, the Society of American Bacteriologists has taken the first steps in the right direction [Winslow *et al.* 1920]. It has appointed a committee on the classification of bacteria which has evolved a framework that may be called successful in many respects, and that consequently deserves the whole-hearted attention of bacteriologists from all over the world. It would be most desirable if some degree of international agreement could soon be reached concerning this matter.

Whereas Rahn, too, calls attention to the example of the U.S.A. as one of the few countries where bacteriology is studied for its own sake, and exhorts his compatriots to follow this lead, he has at the same time made a statement in which we may rejoice. I quote: 'Die Professor Beijerinck's in Delft (Holland) entspricht ebenfalls den Bedürfnissen der theoretischen Bakteriologie.' And also outside Germany

voices are raised complaining about the neglect of microbiology as a science. Let me cite Nicolle, the Director of the Pasteur Institute in Tunisia, who after his visit to Paris recently wrote [Lichtenberger, 1921]: 'Les études microbiologiques se meurent. Le pays qui a produit Pasteur, Duclaux, Laveran, Roux, pour ne pas citer que les plus illustres et qui a recueilli Metchnikoff, laisse, sans en témoigner nul souci, périr une science qui lui a valu jusqu' à présent une belle part de sa gloire'.

If I contemplate this state of affairs, and particularly if I recall Rahn's sober tribute to my great predecessor's work in the field of theoretical bacteriology, then it is inevitable that I close my address by returning to my starting point. Once more shall I express my profound feeling of responsibility towards microbiology, not merely as a subject to be taught at this university, but also as a science. I shall endeavour to serve it according to the best of my modest abilities.

Members of the Board of Trustees,

It is for me a reason of deep-felt gratitude that you have been willing to propose my name to the Government for the chair of microbiology; me who resided at so great a distance from my mother-country, and who must perforce have been a more or less mythical personality to you. To me this is proof that in your choice you have also been guided by considerations of potentialities, which implies confidence in my future efforts and ability. It will be my serious endeavour not to shame this confidence.

Moreover, I want to express the hope that you will not deny me your support. Personally I do not doubt that you also represent that spirit of progressiveness and that keen insight that were exhibited by the educational authorities when, in 1895, they decided to include bacteriology as a subject in the curriculum of the Polytechnical School, a farsightedness which, as I have previously indicated, elicits the envy of other countries even to-day. What I have said may contribute to keeping alive the notion that, amongst the various sciences taught at this university, general and applied microbiology deserves to occupy an important place, at present, and perhaps even more so in future.

Highly esteemed Beijerinck,

Amongst the congratulations that I received as a result of my appoint-

ment none was more valuable to me than your brief announcement that it caused you satisfaction that I had been selected as your successor.

Not yet a year ago authorities whose opinion is of significance also to you have honoured you, 'anerkannten Meister der mikrobiologischen Wissenschaft', as Lindner said, and have attempted to do homage to your merits in connexion with the exemplary and dedicated manner in which you have served this science. A feeble reiteration of such praise from my side cannot be agreeable to you. But here I will proclaim my deep recognition that the occupancy of this chair implies also that at least part of that which your genius and unremitting endeavour has created is now entrusted to my care.

It would be fruitless for me to attempt to maintain the lustre that your institute has acquired by virtue of your work. But to contribute to making this golden quarter century of Dutch microbiology something that will continue to live in the minds of a new generation, that is within my power.

Professors of the Technological University,

On entering upon my duties I wish to assure my colleagues at the Technological University that I greatly appreciate being included in their community. I hope that on occasion I may be permitted to appeal to you for assistance. This applies particularly to the members of the Department of Chemical Technology, amongst whom I may greet many of my former teachers. Although the isolation of micro-organisms may be an important aspect of my future endeavour, isolation of microbiology will never be its aim. I am far too much aware of the fact that our separate fields interpenetrate than that I would not attach great importance to a close co-operation. Allow me to benefit from your wide knowledge and mature experience.

Not in the last place do I think in this connexion of you, dear Böescken. My remarks may have suggested a certain competitiveness between purely chemical and biochemical procedures in industry; but this does not imply a lack of realisation that biochemical problems can be solved only with the aid of organic chemistry. Although something may have been added to our previous relationship of teacher and pupil, I wish nevertheless that in many respects this relationship may be perpetuated.

Dear Van Iterson,

That I, who have been privileged to be your long-time assistant, have now been called upon to succeed him whose assistant you yourself have been for many years, reminds me of the atavistic phenomena that are so characteristic of certain higher plants, to use another biological analogy. To me this is proof of the close relationship that exists between the subjects we are to teach. It seems to me that our teaching will profit if at all times we inculcate a recognition of this relationship into the future chemical engineers. It is for this reason, too, that I here want to express the ardent hope that you may be found willing to grant me your continued support, tutelage, and friendship which you have so lavishly accorded me hitherto. Be convinced that I shall always remember that it was you who showed me the way to independent research. During the nearly six years that have elapsed since I left your laboratory I have realized how much more you have imparted to me for life.

My parents,

My presence here is certainly in no small measure the result of your devoted cares. I feel fortunate that I may publicly thank you for this. The education you gave me was characterized by acts of love rather than by verbose theory. For that very reason I shall not expand this fervent testimony.

Ladies and gentlemen, students of the Technological University,

Even though so little time has gone by since the moment when I left this university, I cannot claim that we are mutually acquainted. It is probable that all of us will have changed too much during that brief span. On the one hand, the war has also caused upheavals in the student community whose significance I cannot yet fathom. On the other hand, fate has taken me to far-away countries, and did not Goethe, in his 'Wahlverwandschaften', say that 'Die Gesinnungen ändern sich gewiss in einem Lande, wo Elefante und Tiger zuhause sind'?

It is true that I encountered neither elephants nor tigers in a free state; but the emancipating influence exerted by life in a foreign environment has not worn off. But, although we may not yet be able to meet as old acquaintances, I should like to express the hope that we may soon have reached that stage.

I wish to address a special message particularly to the prospective chemical engineers. My presence in Delft will probably remain unnoticed by many of you. But to those who, impelled perhaps by an inborn love of living nature, should decide to include in their program the study of microbiology I want to say that, whenever possible, I shall ever try to aid and guide you.

I have already briefly alluded to my sojourn in the beautiful tropical Netherlands. In that environment I have acquired many good friends who will be gratefully remembered. Apart from that, however, I must stress another point. Whoever has been so favoured as to spend, as it was my lot, a number of years in that enchanted state of symbiosis between Europeans and Orientals, recognizes at the time of departure that he has incurred a mental debt of honour. I consider it my duty emphatically to acknowledge this. May it be vouchsafed me to redeem part of this debt by awakening in some of my future pupils an interest in the growing and fermenting communities of the Far East, an interest that at some time will express itself in deeds. And I shall experience a feeling of exultance if their pursuits in that part of the world were to give them a degree of satisfaction such as I was privileged to taste, and if their efforts were to accrue to the benefit of that community.

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UNITY AND DIVERSITY IN THE METABOLISM OF MICRO-ORGANISMS

‘My daughter Alice is a student in High School. One of the prescribed courses is General Science. The section on Bacteria left her with a vague impression of a world teeming with deadly germs awaiting an opportunity to infect mankind. It seems probable that this malignant conception of bacteria is very generally held.

‘In reality civilization owes much to the microbe.’

(From the Preface of A. I. Kendall, ‘Civilisation and the Microbe’. Boston, 1923)

WHEN, some time ago, I received the esteemed invitation to deliver a lecture on some biochemical subject before the Netherlands’ Chemical Society, I gladly accepted because a true microbiologist may never pass up the opportunity to contribute to the vindication of the smallest living organisms. For there is an altogether too prevalent notion that the microbes, as irreconcilable enemies of man, plant, and animal, deserve attention only in order to make it possible the better to combat them. However understandable such a concept may be in view of the beneficial effects resulting from the brilliant discoveries of the rôle of microbes in numerous diseases, it nevertheless does not detract from the fact that it represents an extremely warped picture of reality.

But I shall not use the time at my disposal to show you how, in a microbe-less world, the conditions for human life on earth would soon no longer be realized, so that man possesses at least as many friends as enemies in the domain of the microbes. Taking advantage of the fact that I am addressing a chemically trained audience, I shall rather limit myself to an attempt at striking a sensitive chord by discussing the chemical potentialities of micro-organisms. I trust that a sober review of these capacities may suffice to make you regard these smallest living beings with a little more sympathy in the future.

The most specific characteristic of living matter resides in its metabolic properties. It is an empirical fact that the maintenance of life requires a continuous supply of special chemical substances which, in

the living cell, undergo transformations that lead to their partial excretion in altered form.

You are sufficiently familiar with the notion that this applies to the higher organisms; the idea that it is equally true for the microbes follows immediately from the consideration that in so many cases the presence of microbes is forced upon us by the very fact that we observe chemical changes. When milk turns sour, when sugar- or protein-containing solutions start to ferment, it is first of all metabolic activities that draw our attention, and, after half a century of microbiological research, will lead to the inference that microbes are present.

As the title of to-day's lecture indicates, it is my intention to discuss the unity and diversity in microbial metabolism. But I shall take the liberty of changing the sequence and first to dwell upon the diversity. Later on I shall then try to indicate some aspects of the unity by which this diversity is tied together. In doing so I shall also follow in the main the historical development of microbiology.

When, owing to Pasteur's pioneer investigations, the idea had become established that fermentation, putrefaction, and mineralization were none other than metabolic processes of microscopically small organisms, microbiologists obviously considered it their first duty to make a survey of the multitude of different causative agents.

Chemically the diversity manifested itself in two ways. It was observed on the one hand that one and the same medium might yield a variety of metabolic products; on the other that different microbes differ greatly in their requirements for particular chemical substances. Let me first illustrate the former aspect with some examples.

If I inoculate a series of flasks, each containing a sterile solution of 5 per cent glucose in yeast extract, with pure cultures of a film-forming yeast (*Mycoderma cerevisiae*), and with some common moulds, such as *Aspergillus niger* and *Citromyces glaber*, respectively, a chemical analysis of the cultures at the end of their development will yield different results. Confining the investigation to a determination of the fate of the consumed sugar, it will be found that the film yeast has oxidized it to carbon dioxide and water; hence the over-all metabolism resembles that of animals. In contrast we shall find that *Asp. niger* and *C. glaber* have produced sizable amounts of oxalic and citric acid, respectively, in addition to carbon dioxide. Consequently this simple experiment reveals metabolic differences although these are not as yet very

pronounced. Much more spectacular is the result of a larger experiment in which flasks with the same medium are inoculated with pure cultures of *Saccharomyces cerevisiae* (baker's yeast), *Lactobacillus delbrückii* (the so-called 'domesticated' lactic acid bacterium), *Lactobacillus fermentum* (a 'wild' lactic acid bacterium), *Bacterium coli*, *Bacterium aerogenes*, *Bacterium typhosum*, *Granulobacter saccharobutyricum*, and *Granulobacter butylicum*, and incubated under exclusion of air. After some time it will be evident that the yeast has converted the sugar largely into ethanol and carbon dioxide; *L. delbrückii* into lactic acid; *L. fermentum* into lactic and acetic acids, ethanol and carbon dioxide; *B. coli* into lactic, acetic, and succinic acids, carbon dioxide, and hydrogen; *B. aerogenes* into the same products with, in addition, 2,3-butylene glycol; *B. typhosum* into formic, acetic, and lactic acids, and ethanol; *G. saccharobutyricum* into butyric and acetic acids, carbon dioxide, and hydrogen; *G. butylicum* into butanol, acetone, carbon dioxide, and hydrogen. Thus a remarkable diversity emerges.

Nevertheless, this diversity in products obtainable from a single substrate is almost negligible in comparison with the differences that various microbes exhibit with respect to their nutrient requirements.

For their studies on micro-organisms the early microbiologists depended more or less on the fortuitous appearance of special types. But gradually they became aware of correlations between the initial composition of the medium and the microbes therein encountered. To mention just one example: it soon became clear that true yeasts are found only in sugar media. After the introduction of pure culture methods had permitted a closer investigation of metabolic activities, it became increasingly evident that substances which represent excellent foodstuffs for one type of microbe may be less so, if not entirely unsuitable, for others. Once this had been recognized, the idea obviously occurred that the investigator possessed a powerful tool for encouraging the development of certain microbes at the expense of others present in the inoculum. Although ultimately this method of elective cultures harks back to Pasteur and Raoult, it is in the hands of Beijerinck and Winogradsky that it has indubitably produced the richest harvest. Well-nigh overwhelming is the metabolic diversity revealed by the consistent application of their 'enrichment cultures'; some examples may illustrate this.

Based on Berthelot's studies, showing that the increase in bound

nitrogen of fallow soils was unquestionably the result of a biological process, Winogradsky [1902] succeeded in isolating one of the causative agents, *Clostridium pasteurianum*, by means of the elective culture method in 1893. It turned out to be an organism that can grow only in the absence of air. The following remarkable experiment throws a clear light on its extra-ordinary metabolism.* A solution containing glucose and all other elements necessary for growth with the exception of nitrogen is inoculated with spores of this bacterium. The culture is placed in a container which is thereupon evacuated in order to remove the toxic oxygen. Even after prolonged incubation one does not observe any changes. At this point gaseous nitrogen, freed from the last traces of oxygen and of nitrogenous compounds, is admitted. After 24 hours a vigorous fermentation ensues, and in a few days the sugar has completely disappeared, the bacteria have grown profusely, and nitrogen has been fixed. This demonstrates the remarkable phenomenon of a living organism that is roused to life from a latent state by the inert gaseous nitrogen!

Closely related to this curious organism is the bacterium, *Azotobacter chroococcum*, that was discovered a few years later by Beijerinck and Van Delden. It, too, fixes nitrogen, and to an even greater extent. But in contrast to *C. pasteurianum* it can do so only if an ample supply of oxygen ensures a rapid oxidation of organic substrates.**

Is it surprising that the activities of these organisms have elicited the envy of men like Haber?

Yet other specialists among bacteria demand our attention. How curious a group of organisms are not the urea bacteria that more or less intensively convert urea into ammonium carbonate! Special mention deserves *Urobacillus pasteurii* which under suitable conditions com-

* In spite of the fact that the assimilation of gaseous nitrogen by *Cl. pasteurianum* has long been established beyond any doubt, it is, as far as I am aware, only recently that the elegant demonstration has been devised (by H. J. L. Donker, Assistant at the Laboratory of Microbiology of the Technological University) which illustrates the sharp contrast between the harmful influence of oxygen and the salutary effect of nitrogen gas. For the benefit of those who might wish to repeat this experiment, it must be mentioned that the addition of a small amount of humate to the culture medium is indispensable for its success. This does not affect the sense of the experiment.

** For additional details of the organisms studied by Beijerinck, reference may be made to his 'Collected Works', published in 1922.

pletely hydrolyses a 10 per cent urea solution and thus can tolerate with impunity the alkaline reaction of a 16 per cent ammonium carbonate solution.

I may not omit an organism such as *Bacillus oligocarophilus*, for which Beijerinck and Van Delden showed that it can derive its nutrients from the traces of organic matter that never seem to be lacking in laboratory air.* Also the group of bacteria that feed on hydrocarbon vapours, studied by Söhngen [1903], may not be neglected.

But even this diversity is still limited in one respect. The carbon, essential for the growth of the organisms so far mentioned, must be supplied in the form of an organic substance, though the nature of the latter may vary enormously. This implies that in the end these organisms derive their food from other organisms, hence their designation as heterotrophs.

Nevertheless, it is almost forty years ago since Winogradsky expressed the idea that even in this regard the diversity of microbial metabolism is not limited. To be sure, it had long ago been established that chlorophyll-containing microbes could build up their cellular constituents from carbon dioxide and other minerals, by virtue of the absorbed solar energy. But that colourless microbes, without the aid of radiant energy, could do so too is a concept so daring that even to-day it is surprising that a human mind has ventured to propose it.

A genius like Winogradsky [1887] did not hesitate, however, to conclude, as early as 1887, from his simple and ingenious experiments that the colourless *Beggiatoa alba*, frequently encountered in sulphur spring waters, can grow in darkness in a strictly mineral medium. The organic cellular constituents would be synthesized from carbon dioxide, a process made possible because the necessary energy would be obtained from the oxidation of hydrogen sulphide, first to sulphur, and next to sulphate. In addition to *Beggiatoa* this would also apply to a large group of bacteria, partly colourless, partly purple, that exhibit the common characteristic of depositing free sulphur in their cells.

Subsequent experiments have amply confirmed the correctness of Winogradsky's concept. Consciously executed elective cultures have shown that, in addition to the relatively large organisms that deposit

* Later investigators are inclined primarily to implicate traces of carbon monoxide, a gas that is extremely toxic for higher animals, but can certainly be used as the sole carbon source by *B. oligocarophilus*. See: Centr. Bakt. II, 57, 309, 1922.

colloidal sulphur in the form of droplets and as a reserve material in their cells, and which had long been known to naturalists, there exists another large group of small bacteria that, though producing sulphur extra-cellularly when grown in sulphide media, yet must be reckoned as belonging to the physiological group of sulphur bacteria on account of their metabolic behaviour which is similar to that of *Beggiatoa*. These bacteria, discovered by Nathansohn, but studied more particularly by Beijerinck, Lieske [1912], and Jacobsen [1912, 1914], have recently been much discussed in America, and permit me to give you another striking example of the chemical proficiency of the microbes. In a series of papers the American microbiologist, Waksman [1922], has reported on the astounding properties of an organism, designated as *Thiobacillus thiooxidans*, that can also oxidize sulphur powder to sulphuric acid, and get along with carbon dioxide as the sole carbon source. It differs from *Thiobacillus thioparus*, earlier described by Beijerinck and by Jacobsen, especially by its insensitivity to acid which is so pronounced that in certain media the pH reaches 0.6. Nay, J. G. Lipman, the well-known editor-in-chief of 'Soil Science', has communicated in a recent paper that *T. thiooxidans* can still effect a perceptible sulphur oxidation in solutions containing 5 per cent sulphuric acid.

It should not surprise you that this powerful ability to produce sulphuric acid has been exploited in different directions in the U.S.A. I shall mention but one example because, in a sense, it implies an attack on one of the branches of chemical industry. Lipman and Waksman have already carried out experiments on a rather extensive scale to test the possibility of circumventing the commercial manufacture of superphosphate by fertilizing the soil with a mixture of natural phosphate and free sulphur, and thus causing the localized formation of sulphuric acid, hence also of superphosphate, through the activity of *T. thiooxidans*. In certain soils this treatment appears to have given fully satisfactory results.

As far as an impressive chemical performance is concerned the last-mentioned bacterium is still exceeded, however, by Beijerinck's *T. denitrificans* which can grow in the complete absence of oxygen and gaseous carbon dioxide in a medium containing sulphur, potassium nitrate, chalk, and a small amount of phosphate; thus it should be able to produce organic matter from these compounds in deeper soil layers.

I could display before you several other specialists which resemble the sulphur bacteria in being able to grow in purely mineral media. Time limitations prevent me from dwelling on them; I shall merely mention some other examples, such as the bacteria that oxidize nitrite to nitrate, or ammonia to nitrite, and for which glucose is almost as toxic as is sublimate for other organisms. Let me finally also refer to the various types of organisms for which mixtures of hydrogen and oxygen, methane and oxygen, and hydrogen and nitrous oxide constitute nutrients.

If we consider all these data it is well-nigh impossible not to be impressed by the enormous diversity in microbial metabolism. If we further survey the trends in microbiology during the past few decennia it is hard to avoid the conclusion that the attempts to demonstrate this diversity have largely dominated the investigations. It was not so much the microbes themselves that were the starting point for the studies; rather was the mere suspicion that certain chemical transformations might occur in nature a sufficient impetus to formulate the hypothesis that there would be microbes to accelerate them. And the correctness of the hypothesis was substantiated in nearly every case by those who had learned to utilize the elective culture method. The very consideration that the cycle of matter on earth is closed, and that consequently the naturally occurring hydrocarbons must eventually be converted into carbon dioxide and water, led Söhnngen to the discovery and isolation of an important group of bacteria, members of the genus *Mycobacterium*, that can use these substances as carbon source.

I can cite an even more striking instance. The studies of the above-mentioned autotrophic bacteria, of which the sulphur-, nitrite-, and ammonia-oxidizing bacteria are the best-known examples, unconsciously led Nathansohn to the bold hypothesis that still other reactions, proceeding very slowly at ordinary temperatures, might serve as a basis for microbial metabolism even if the oxidizable compound is not known to occur in nature. Thus he could ascertain the fact that the exclusively man-made thiosulphate can serve as the major nutrient for particular types of sulphur bacteria.

These examples may suffice to show how gradually the investigations became subservient to what might be called a 'microbiological imperialism'. It became a contest to open up new, seemingly inacces-

sible areas for the microbes. Barely had one specialist among micro-organisms been discovered when another even more spectacular one was announced; the microbiological theatre resembled one grand naturalistic vaudeville show.

However great may be our admiration for those who, through their intuition and ingenious experimentation, have guided this flight of general microbiological discoveries, and however indispensable has been this thorough exploration of the microbial world for the continued development of microbiology, in the long run this approach could not persistently be satisfying. Even while the main current of microbiological research was concentrated on the diversity, a field of study gradually developed in which a search for unity in the diversity became apparent. It would be unjust to depict this trend as an entirely new and recent development. Far from it: many classical figures in microbiological science, and its founder, Pasteur, in the very first place, must receive honourable mention in this connexion. Nevertheless, one may safely say that the attempt to view microbial metabolism in the light of the outcome of general physiological research has been deliberately initiated by the German physiologist and hygienist Rubner, only at the beginning of the twentieth century. And we may immediately add that thus far this approach has found very little response. This regrettable fact can be readily explained by the circumstance that microbiology has hitherto developed primarily as an applied science. By far the majority of medical, technological, and agricultural microbiologists have studied the rôle of microbes in the important and eminently practical problems they had to face. And of the relatively very small number of investigators who did have the opportunity and desire to study microbes for their own sake, the majority was under the spell of the diversity; quite understandably so in view of the fascination of this type of work.

If now I am going to embark on the attempt to give you a glimpse of the unity that can be discovered in microbial metabolism, I do so with the full knowledge that it is but a meagre account I have to offer. That nevertheless I have ventured to solicit your attention for this topic finds its explanation in the fact that I shall thus have the opportunity to make you realize that this still presents an immense field of endeavour. It seems to me that the solution of the problems in

this area is indispensable to elevate microbiology from its status as a largely descriptive branch of science to a higher plane, and equally so to the application of microbiology to many situations. And I am firmly convinced that only a close cooperation between microbiologists and accomplished chemists can lead to advances in this respect.

Let me then first of all show you how the unity in the divergent metabolic processes of the microbes finds expression in the fact that we recognise in them the same general trends that have come to light as a result of investigations of the metabolism of higher organisms. Without discussing this aspect in detail, the following remarks may serve to illustrate this point. Studies of the metabolism of higher organisms have unequivocally shown that one can always distinguish two types of processes. Part of the foodstuffs is converted into cell materials, the latter being used either for growth or for replacement of degenerated cellular constituents. Another fraction of the food appears to owe its significance largely to the therein accumulated chemical energy which is unleashed by the living cell, thus enabling it to carry out energy-requiring functions; this part is ultimately degraded, generally producing heat. These two processes are differentiated as assimilation and dissimulation.*

Doubtless the dissimilatory process is the more essential characteristic of life. Whereas other typical vital phenomena, such as growth, reproduction, internal or external motion, may frequently be lacking, dissimulation is absent only in some stages of latent life, resulting, for example, from desiccation.

Consequently it had been established that in the case of higher organisms the perpetuation of life is tied to a continuous conversion of chemical into other forms of energy, and Rubner demonstrated that the first law of thermodynamics applies to the energy transformations in the animal body, while Atwater showed this also for man.

As far as the nature of the energy-providing reactions is concerned, Lavoisier had already pointed to the significance of the respiratory process, *i.e.*, the slow combustion taking place in living organisms,

* Perhaps the terms 'Bau-' and 'Betriebsstoffwechsel', customarily used in the German literature, make for a clearer distinction. For a further consideration of these problems see, *e.g.*, M. Rubner, 'Kraft und Stoff im Haushalte der Natur' (1909), and the chapter by C. Oppenheimer, 'Energetik der lebenden Substanz', in his 'Handb. d. Biochemie d. Menschen u.d. Tiere', 2nd ed., Vol. II, p. 223, 1923.

which reveals itself through their requirement for oxygen. The validity of this concept was emphasized when Rubner experimentally established his principle of 'isodynamic substitution'. He showed that, up to a point, proteins, fats, and carbohydrates can replace one another, in a weight ratio that is inversely proportional to the heat of combustion of these substances, in the adult animal for the maintenance of the same vital functions; in other words that, within certain limits, equality in chemical energy corresponds to equality in food value.

As early as 1885 Rubner pointed out that in all probability it would be possible that also in the metabolism of micro-organisms a conversion could be designated which derives its significance for the organism entirely from the resultant energy liberation. Now Pasteur, who, in 1860, had discovered the first instance of organisms that can multiply in the complete absence of oxygen, had intuitively recognized the connexion between the absence of respiration in these organisms and the fermentation process that characterizes their mode of life.

Meanwhile the mutual substitution of respiration and fermentation was initially considered largely from a material angle, so that the term fermentation was used to indicate a respiration with bound oxygen. The need to consider this substitution primarily on the basis of energetics was first formulated with sufficient clarity by Rubner as a logical consequence of his attempt to interpret the metabolism of any and all living creatures from a single point of view. But not until 1902 did he begin his series of micro-calorimetric measurements of the metabolism of various microbes which corroborated the validity of these hypotheses. Since that time it has been satisfactorily established that the metabolic activities of every microbe comprise the processes whereby new cell material is synthesized, as well as dissimilatory processes characterized by the fact that chemical energy is utilized by the living cell for its energy-requiring functions, and finally appears as heat.

A number of the most important dissimilatory processes encountered among various groups of micro-organisms is summarized in Table I.*

* In computing the majority of the caloric effects listed in this Table, I have been privileged to profit by the authoritative advice of my colleague, P. E. Verkade, to whom, here too, I want to express my sincere gratitude. It may be mentioned that an attempt has been made to calculate, as accurately as possible, the differences between the heats of combustion of the aqueous solutions of the substrates and of the metabolic products.

TABLE I. MICROBIAL DISSIMILATORY PROCESSES*

A. Oxydative processes	Examples of organisms:
1. $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 676 \text{ cal.}$	various fungi
2. $C_2H_5OH + 3O_2 = 2CO_2 + 3H_2O + 325\frac{1}{2} \text{ cal.}$	mycodermic yeasts (‘Kahmhefen’)
3. $CH_2NH_2COOH + 1\frac{1}{2}O_2 = 2CO_2 + H_2O + NH_3 + 142 \text{ cal.}$	many aerobic bacteria
4. $C_2H_5OH + O_2 = CH_3COOH + H_2O + 116\frac{1}{2} \text{ cal.}$	acetic acid bacteria
5. $CH_4 + 2O_2 = CO_2 + 2H_2O + 210 \text{ cal.}$	methane oxidizing bacteria
6. $H_2 + \frac{1}{2}O_2 = H_2O + 68\frac{1}{2} \text{ cal.}$	hydrogen oxidizing bacteria
7. $NH_3 + 1\frac{1}{2}O_2 = HNO_2 + H_2O + 79 \text{ cal.}$	nitrite forming bacteria
8. $KNO_2 + \frac{1}{2}O_2 = KNO_3 + 22 \text{ cal.}$	nitrate forming bacteria
9. $H_2S + \frac{1}{2}O_2 = H_2O + S + 67 \text{ cal.}$	sulphur bacteria
10. $S + 1\frac{1}{2}O_2 + H_2O = H_2SO_4 + 141 \text{ cal.}$	sulphur bacteria
B. Fermentative processes	
11. $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 25 \text{ cal.}$	yeasts
12. $C_6H_{12}O_6 = 2C_3H_6O_3 + 28 \text{ cal.}$	lactic acid bacteria
13. $C_6H_{12}O_6 = C_4H_8O_2 + 2CO_2 + 2H_2 + 15 \text{ cal.}$	butyric acid bacteria
14. $C_6H_{12}O_6 + \frac{1}{2}H_2O = CH_3CHOHCOOH + CO_2 + H_2 + \frac{1}{2}CH_3COOH + \frac{1}{2}C_2H_5OH + 8 \text{ cal.}$	coliform bacteria
15. $(CH_3COO)_2Ca + H_2O = CaCO_3 + CO_2 + 2CH_4 + 19 \text{ cal.}$	methane bacteria
16. $CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3 + 6 \text{ cal.}$	urea splitting bacteria
17. $C_2H_5OH + 2.4KNO_3 = 1.2N_2 + 1.2K_2CO_3 + 0.8CO_2 + 3H_2O + 293 \text{ cal.}$	denitrifying bacteria
18. $C_2H_5OH + 1\frac{1}{2}CaSO_4 = 1\frac{1}{2}H_2S + 1\frac{1}{2}CaCO_3 + \frac{1}{2}CO_2 + 1\frac{1}{2}H_2O + 14 \text{ cal.}$	sulphate reducing bacteria

* This Table makes no attempt whatever at completeness. In particular, the list of oxidative dissimilation processes with organic substrates can be expanded indefinitely. As for the fermentative dissimilation processes, the most important types known today are represented, with the exception of the anaerobic decomposition of protein breakdown products. These have been omitted because it is very difficult to denote them by means of simple equations. See, however, Arch. f. Hyg., 66, 209, 1908.

The first ten represent oxidative dissimilation reactions of which the upper three occur also in higher animals, the others being typical dissimilatory processes of the earlier mentioned microbes. The dissimilations listed under the heading 'fermentative' are examples of transformations that satisfy the energetic requirements of organisms that live temporarily or permanently in the absence of oxygen.

These fermentative processes, understandably, are characterized by a considerably smaller caloric effect, and the correctness of the energetic approach to these transformations is reflected in the fact that per unit weight of the causative organism comparatively much more food is used, and much greater amounts of metabolic products are formed during fermentative existence. Particularly clearly is this phenomenon revealed by organisms which, depending on circumstances, derive their energy either from an oxidative or from a fermentative dissimilation. This is true, for example, for yeast; and Pasteur had already established that the sugar consumption per unit weight is considerably greater during anaerobic than during aerobic cultivation; in the latter case part of the sugar is also oxidized.

Now it cannot be doubted that the energetic interpretation of metabolism can also be used for the clarification and systematization in other directions. In the first place it forces the investigator to develop a clearer picture than is customary of the metabolism of the organism studied. To mention an example: it is usually stated without further specification that the extensively investigated *B. coli* can grow in meat extract broth both with and without sugar, and that it is a facultative anaerobe, implying that it can develop both in the presence and in the absence of oxygen. Many a microbiologist does not realize, however, that the dissimilation of this bacterium consists in an oxidative degradation of proteinaceous split products on the one hand, but in a fermentative decomposition of carbohydrates or sugar alcohols on the other, so that the presence of the last-mentioned substances is a prerequisite for anaerobic growth. This is in contrast to bacteria of the *Proteus*-group which are usually similarly characterized in the literature, although they appear to possess the property of obtaining energy from a fermentation of protein degradation products so that they can lead an anaerobic existence even in the absence of carbohydrates. While this example indicates that the vague designation of an organism as a facultative anaerobe should be replaced by a rational con-

sideration of potential dissimilation processes in order to permit a proper insight into the conditions needed for development, the following example may further illustrate this.

The organisms that can be assembled in the group of genuine lactic acid bacteria are often designated as facultatively anaerobic. In a sense this is permissible because the majority can indeed develop in the presence as well as in the absence of air. It would, however, be a glaring error if one were to infer from this fact that these organisms, like the above-mentioned facultative anaerobes, can display both an oxidative and a fermentative metabolism. On the contrary, even in the presence of air the lactic acid bacteria do not utilize oxygen in their metabolism. They are therefore purely fermentative organisms; carbohydrates or sugar alcohols are essential for their growth; and they differ from other strictly fermentative microbes only in the fact that free oxygen does not completely inhibit their development.

In this connexion it is not without significance to point out that the absence of an oxidative metabolism among the lactic acid bacteria is undoubtedly related to a property that Beijerinck in 1893 established for all lactic acid bacteria, *viz.*, the absence of the enzyme catalase which decomposes hydrogen peroxide into water and oxygen, and which had been found in all cells of higher plants and animals, and until then also in all microbes that had been tested. Later Orla-Jensen pointed out that the exclusively anaerobic butyric acid bacteria also lack catalase, and we have established the same situation in the case of anaerobic protein-decomposing bacteria. The widely accepted, though still hotly disputed [Dakin 1922] hypothesis that in all aerobic organisms, the higher as well as the lower, catalase plays a rôle in the transfer of oxygen to the oxidizable substrate is undoubtedly supported by these facts. Conversely it seems likely that a study of the occurrence of catalase in a newly isolated microbe can be fruitfully employed to determine the presence of an oxidative metabolism.

The great significance of the establishment of the nature of a microbe's dissimilation processes, these primarily characterizing its metabolism, also resides in the fact that the natural relationships of micro-organisms are unquestionably expressed in their metabolism.*

* But – as Orla-Jensen too points out correctly – it does not follow that identical metabolic processes may not also be encountered among phylogenetically independent evolutionary lines.

















This idea has been used in an eminent manner by Orla-Jensen [1909] in his sketch of a general system of classification of bacteria. By assigning every microbe to one of the natural groups, whose delineations become progressively clearer, it will frequently be possible to predict its properties on the basis of its relationships, and it will also become feasible to indicate more rational culture conditions for it. For example, one frequently hears complaints concerning the difficulty of cultivating numerous streptococci; this must in the first place be ascribed to the fact that the investigators fail to realize that they are working with organisms belonging to the group of genuine lactic acid bacteria.

Table II indicates the distribution of dissimilatory processes among some important groups of microbes. It shows that, as a rule, oxidative processes do not exhibit any great specificity as far as the nature of the oxidizable substrate is concerned. Not infrequently the oxidizing capacity extends to nitrogen-containing compounds, to carbohydrates and sugar alcohols, and to organic acids, although in the series, acetic acid bacteria, moulds, aerobic sporeformers, and the *Pseudomonas* group,* a decreasing tendency towards carbohydrate oxidation and an increasing one towards the oxidation of nitrogen-containing compounds may be detected. In contrast, the fermentative processes are generally more dependent on a specific substrate, even though in some groups several potentialities may co-exist.

When further considering the meaning of the energetic aspects of metabolism we are led to still other notions. That man or a higher animal requires energy is a notion that we immediately seem to recognize as befitting. The maintenance of the body temperature, the performance of internal and external work, these are inconceivable without energy supply. But if one raises the question what function is served by the continuous flow of energy which, according to experience, is requisite for the perpetuation of life even of a non-motile microbe, maintained at constant temperature, and not displaying any internal movements, there appear to be only two rational answers. On the one hand we may assume that the energy conversion is simply a necessary condition for the living substance as such; on the other hand it is tempting to postulate that there exists a close connexion between

* Although the organisms assembled in this group exhibit physiological similarities, they do not form a 'natural group'; see the previous footnote.


TABLE II. DISSIMILATORY PROCESSES OF SOME IMPORTANT GROUPS OF MICRO-ORGANISMS

Oxidative dissimilatory processes		Fermentative dissimilatory processes	
	autotrophic bacteria		
	acetic acid bacteria		yeasts
	fungi		coliform bacteria
	aerobic sporeforming bacteria		bacteria of the Proteus group
			urea splitting bacteria
	Pseudomonas Micrococcus Sarcina Spirillum Vibrio Mycobacterium?		denitrifying bacteria
			lactic acid bacteria
			anaerobic cellulose decomposing bacteria
			butyric acid bacteria
			anaerobic protein fermenting bacteria
			methane bacteria
			sulfate reducing bacteria

 inorganic compounds

 organic substances containing nitrogen

 carbohydrates and sugaralcohols

 salts of organic acid

this incessant utilization of chemical energy and the other side of metabolism, *viz.*, the assimilation, or the production of new cell constituents.

In contemplating this possibility the problem arises whether such a situation should not be apparent if one were to succeed in reducing the extent of assimilation by a microbe to a minimum, so that the visual new-formation of cell material, *i.e.* multiplication, is excluded. We then encounter the problem whether it is possible to maintain microbial cells alive by the supply of a food ration that will just prevent multiplication. Rubner [1913] has made an attempt at experimentally testing this possibility with yeast, and had concluded that it does not exist. One may, however, raise serious objections against the technique he used, so that we can only state that in the realm of microbes this fundamental problem is still unsolved.

But even in those cases where visual growth fails to take place we must always count with the occurrence of a process of replacement of worn-out cell materials, so that it remains possible to regard the function of dissimilation as one serving assimilation. On the other hand it is patently true that at least part of the dissimilatory energy is indispensable for assimilation whenever we can ascertain that the latter is accompanied by an obvious increase in energy. A good example of such a situation is provided by the autotrophic bacteria which produce organic matter, such as bacterial protein, from carbon dioxide. In the case of such organisms, sometimes improperly designated as chemosynthetic, the microbiologists have consequently been wont to stress the energy-providing reactions.

If it is remembered, however, that experience has shown how, for example, the sulphur bacteria must oxidise 32 g of sulphur to sulphuric acid in order to fix 1 g of carbon in the form of bacterial cells (dissimilation process 10, Table I), it is immediately apparent that the autotrophic bacteria, too, retain only a small fraction of the dissimilatory energy in the form of assimilation products. This point is still more obvious in the case of microbes that depend on organic nutrients. Rubner's calorimetric measurements have shown convincingly that the autolysis of yeast cells, like most enzymatic hydrolyses, proceeds with a very small heat production. Conversely it must therefore be true that the formation of yeast cells from the products of autolysis implies an equally small energy fixation. Nevertheless, the energy

utilization of the dissimilation that accompanies the reconstitution of yeast in an advanced state of autolysis by the sudden addition of sugar is quite considerable. On this basis one would again be inclined to conclude that the formation of cell constituents represents a process that occurs with a very low energy efficiency, in other words, that the energy derived from some chemical transformation can co-operate in a second reaction only if concomitantly a considerable part of the energy is dissipated as heat.

If now we try to formulate this problem more succinctly, it must be stated that the metabolism of a living cell, which presumably proceeds isothermically, shows us a reaction that leads to an increase in free energy, *viz.*, the assimilation, which obviously is conceivable only if concomitantly a second reaction occurs which proceeds with a larger loss of free energy, so that the free energy of the entire system decreases considerably. Hence I should like to ask the physical chemists amongst my audience whether they know of similar cases of coupled reactions in inanimate systems, and in how far they would have fundamental objections to accepting the occurrence of such energetic coupling.

Euler and Af Ugglas [1911] have posed this question as early as 1911, though perhaps in a less pregnant form. They believe that such energetic interplay between two materially independent reactions is conceivable if both reactions are accelerated by one and the same catalyst. They elucidate this by referring to displacements in equilibria that must necessarily happen if the catalyst forms compounds with the two substrates as well as with the reaction products. Without here entering into a detailed evaluation, it follows from the necessity to assume a coupling of dissimilation and assimilation that it is entirely inconceivable that the energy-yielding dissimilatory process would occur under the influence of a catalyst that can be divorced from the remainder of the living cell. Such a concept, advocated rather thoughtlessly by chemists after Buchner's discovery of zymase, can only be defended by those who are blind to the biological significance of fermentation as the dissimilatory process of the yeast, and which consequently has to be considered in connexion with the assimilation.

That the dissimilatory reactions, in contrast to the preparatory conversions of foodstuffs, occupy a special position in the cell is clearly reflected by the fact that the latter occur under the influence of en-

zymes that frequently can be quantitatively separated from the cells, whereas the former have tenaciously resisted the efforts to isolate the causative enzymes. It is true that Buchner has succeeded in extracting a very small fraction of the fermenting capacity of some specific yeasts from the cells, but various arguments can be adduced in favour of the statement that in those instances the prominent dissimilation catalysed by the living protoplasm is accompanied by a weak sugar fermentation proceeding under the influence of a zymase function that can be isolated in small amounts. This concept is strengthened by the rather ridiculously low yield that Buchner and his collaborators, Gaunt and Meisenheimer [1906], obtained in their attempts to isolate a lactozymase and an alcoholoxidase from lactic and acetic acid bacteria, respectively. In spite of this, they interpret their results as supporting their enzymatic theory of metabolism. Similarly, the old controversy as to whether the urea decomposition by urea bacteria does or does not proceed under the influence of a soluble enzyme, urease, thus appears in a new light. From the genuine urea bacteria, for which the decomposition of urea indubitably is also a dissimilatory process, a separation of urease is practically impossible, as Beijerinck has already shown. In other organisms the splitting of urea appears virtually to have lost its energetic significance, in view of the occurrence of other dissimilatory processes. Thus it becomes understandable that Jacoby [1917, 1923] succeeded in isolating a soluble urease from *Proteus* bacteria; this he would certainly not have accomplished had he used *Urobacillus pasteurii*.*

I do not have the opportunity to dwell much longer on the concepts that arise out of the energetic considerations of metabolism. That it would undoubtedly be extremely rewarding to submit the entire problem of microbial metabolism to a renewed study in connexion with the second law of thermodynamics may here be hinted at. The great significance of this problem for general physiology has been pointed out by Zwaardemaker [1906]. Application of these principles to the relatively simple situation in microbes has hardly been begun thus far; nevertheless, it should almost inevitably be productive of significant results.

* The ready solubility of soybean urease is undoubtedly closely linked to the fact that the decomposition of urea is energetically insignificant for the cells of higher plants because their mode of life is predominantly oxidative.

It is extremely tempting, for instance, to try to establish a relation between the quantitative values of the dissimilation reactions of the various microbes and their characteristic assimilatory processes. Should it not be possible that in this way one might learn to understand why the butyric acid fermentation as dissimilatory process enables the butyric acid bacteria to build up their proteins from ammonia nitrogen, whereas the lactic acid fermentation does not permit the lactic acid bacteria to accomplish this, though they can use peptones for this purpose? It is evident that the caloric effects of the dissimilation processes do not provide an adequate yardstick in this respect, but that it is necessary to determine the free energy decrease of these processes. It may be pointed out by the way that for this reason one may easily conceive of the existence of organisms whose dissimilatory reactions represent spontaneously occurring endothermic processes, causing them, in contrast to all known organisms, not to produce heat but rather to absorb this from their surroundings.

Baron and Polanyi [1913] have pointed out that in the case of reactions that proceed with a small caloric effect the decrease in free energy by no means agrees with the caloric effect, whereas in oxidative processes the discrepancy between the two is not very great. If this be so, and if we must also conclude that the dissimilatory energy conversions are used for assimilation, then we might expect that for one and the same organism, or for closely related types, an identical dissimilatory energy conversion should express itself in identical assimilatory effects. The opportunity to check this is offered by the ammonia- and nitrite-oxidizing bacteria, organisms with very similar metabolic properties but which derive their energy from dissimilations with different caloric effects. Now it is no doubt interesting that, while the caloric effects of these reactions are 79 and 22, the ratios of nitrogen oxidized to carbon assimilated by the two organisms are 35/1 and 135/1, respectively. This, therefore, agrees with the expectation that they would be inversely proportional to the caloric effects.

I have, however, lingered too long in discussing the energetic unity in metabolism. It would please me if you had gained the impression that the energetic approach may already open up some attractive vistas, but that a closer investigation has hardly been begun thus far.

It is not only in energetic respect that we may discern a unity in

the metabolism of micro-organisms. Also in material respect there exists a much greater unity than was assumed not so long ago. This has been shown by recent studies; again, the circumstances do not permit me to document this statement extensively. But it may be pointed out that the investigations of Neuberg and collaborators have made it very likely that acetaldehyde occurs as an intermediate product in the fermentations provoked by yeast [cf. Fuchs 1922], coli bacteria [Neuberg and Nord 1919], butyric acid bacteria [Neuberg and Arinstein 1921], and cellulose decomposers [Neuberg and Cohn 1923]. Furthermore, this intermediate product arises in butyric acid fermentations both of substrates with 6 and with 3 carbon atoms per molecule, which at least renders the formation of butyric acid with its 4 carbon atoms from compounds of the C_3 type somewhat more intelligible. The occurrence of pyruvic acid as an intermediate product in diverse fermentations is equally suitable to demonstrate the unity in material respect of at first sight very different processes.

Even stronger does the material unity in metabolic reactions appear if now we consider the microbes that are characterized by a typically oxidative dissimilation. It is evident from Table II that this manner of fulfilling the energetic requirements, characteristic of all higher plants and animals, is also encountered amongst rather divergent groups of microbes. In contrast to the relatively extensive chemical investigations of fermentative processes, these aerobic decompositions have not yet been much studied, however. The explanation for this situation lies at hand; in general, aerobic organisms utilize the energy of the proffered foodstuffs to the maximum extent, that is to say that the oxidation is carried as far as possible, and the food is oxidized to carbon dioxide and water, accompanied by ammonia in the case of the oxidation of nitrogenous substrates.

Nevertheless, exceptions to this rule have long been known. When acetic acid bacteria had been discovered, it was immediately apparent that they provided an instance of organisms characterized by an incomplete oxidative metabolism. This manifested itself not merely in the incomplete oxidation of alcohol to acetic acid, but also of glucose to gluconic acid. Among other groups of aerobic organisms, too, some specialists gradually appeared. Thus the mould, *Aspergillus niger*, acquired some reputation as the causative agent of what has unfortunately been called an oxalic acid fermentation; it was found that if

the organism is grown on sugar-rich media, considerable quantities of oxalic acid were formed. A number of other moulds, closely related to the well-known *Penicillium glaucum*, appeared to produce citric acid under similar conditions; this has been considered a sufficient reason for collecting these specialists in a separate genus, *Citromyces*. Moreover, as early as 1886 the French chemist, Boutroux [1886], had discovered a conversion of glucose, in the presence of calcium carbonate, into calcium keto-gluconate under the influence of an aerobic bacterium that was not described. And around 1900 appeared the publications of Bertrand [1904] on the biochemistry of the so-called sorbose bacterium (*Acetobacter xylinum*) which rightly caused a sensation also amongst organic chemists. Although from the start it was obvious that this bacterium should be classed with the acetic acid bacteria, its metabolism showed nonetheless important deviations from that of the bacteria used in the manufacture of vinegar. This appeared, for instance, from the fact that it produced large amounts of sorbose when cultivated in the presence of sorbitol; mannitol yielded fructose; and glycerol dihydroxyacetone. All of these represent conversions that the ordinary acetic acid bacteria did not effect.

Hence a number of specialists had gradually begun to stand out amidst the at first sight metabolically quite uniform group of aerobic microbes. And the discoverers of these organisms did not tire of singing their praise.

Now it is certainly most interesting that recent investigations have shown that the performances of all these apparently so diverse specialists may be correlated. Firstly, the experiments of Currie [1917], and particularly those of Molliard [1920, 1922], showed that by changing the culture conditions it is possible at will to cause *A. niger* to convert an important fraction of the sugar into gluconic, or citric, or oxalic acid. Consequently one could make this organism, hitherto known only as an oxalic acid specialist, produce substances that until that time had been known only as specific products of acetic acid bacteria, and of *Citromyces* species, respectively. This very fact made it quite likely that the above-mentioned compounds are none other than intermediate products of the oxidative degradation of glucose to carbon dioxide and water. The difference between the specialists would then merely consist in the fact that one organism would stop at an earlier intermediate stage.

Perhaps it may appear to you that the interpretation has always lain at hand that metabolic products such as citric, oxalic, and obviously gluconic acids are normal metabolic products in the oxidation of glucose. This notion had not been generally accepted, however. This appears from the fact that Butkewitsch [1922, 1923] who, during the past several years, has carried out extensive experimental investigations on citric and oxalic acid formation by moulds, has independently reached the same conclusion only quite recently. Experiments carried out in the Delft laboratory during the last year on the metabolism of various acetic acid bacteria have also led to the same concept of a stepwise oxidative degradation. To begin with, De Leeuw probably recovered the bacterium used by Boutroux, and long since lost; it turned out to be an acetic acid bacterium which we have called *Acetobacter suboxydans*. This organism also shows a close relationship to Bertrand's sorbose bacterium, although it clearly differs from the latter in some respects. The investigations have shown that *A. suboxydans* can carry out the same mild oxidations of different sugar alcohols as have already been mentioned in connexion with the sorbose bacterium. But it also appeared that *A. suboxydans* possesses a still weaker oxidative capacity than *A. xylinum*, as evidenced, for example, by the fact that the former oxidizes calcium gluconate only to ketogluconate, while the latter can also oxidize the gluconate to carbonate. Besides, *A. suboxydans*, in contrast to *A. xylinum*, cannot be induced to oxidize substances like dihydroxyacetone and potassium ketogluconate. Thus the latter bacterium appears capable of further oxidizing its characteristic metabolic products under suitable conditions. And this shows up the intermediate nature of these incomplete oxidations.

Still more clearly does the correctness of these concepts follow from the fact that we [Kluyver and De Leeuw 1924] have succeeded in inducing acetic acid bacteria that under ordinary culture conditions completely oxidize substrates like glycerol and mannitol to bring about incomplete oxidations by the use of methods similar to those employed by Molliard in his studies on *Asp. niger*. A few words may here be devoted to a discussion of these methods through which oxidations may be halted at various intermediate stages. Molliard paid attention primarily to the quantitative regulation of the amounts of N, P, and K in the culture medium. In connexion with earlier observations of Beijerinck and Hoyer, we made use of the fact that the

nature of the nitrogenous foodstuffs often determines whether a particular substrate, hence also a particular intermediate product, will or will not be further oxidized. This also reveals a very striking relation between dissimilation and assimilation, and perhaps may open a way for penetrating more deeply into these problems. After all, it is quite remarkable that *A. suboxydans* can grow perfectly well in a mineral medium containing a few per cent mannitol if nitrogen is supplied in the form of an ammonium salt. It is thus certain that this bacterium can use ammonia nitrogen for the synthesis of its proteins. But it appears also that this organism does not grow in the same medium if glucose is substituted for mannitol, whereas, in the presence of peptone, glucose is very rapidly oxidized, and the bacteria then multiply profusely. Glucose is therefore fully adequate as an energy-providing substrate. Thus we see how two substances, each one utilizable in its own function, can furnish an inadequate combination as food. Might it perhaps be possible to ascribe this unexpected phenomenon to a difference in the decrease in free energy of the first stages of the oxidation of mannitol and glucose, respectively, a difference that might cause ammonia nitrogen to be used for the synthesis of bacterial protein in the one, but not in the other case? We don't know; but we can learn from this example that the occurrence of a biological oxidation does not depend only on the nature and condition of the living cells and the presence of an oxidizable substrate, but that it is conditioned by very subtle modifications in the composition of the medium.

A further consideration of these problems inevitably reminds us of those other phenomena of incomplete and insufficient biological oxidations that are characteristic of the pathological deviations in the metabolism of man and animals known as diabetes. Is it a tenuous comparison if we say that we cure *A. suboxydans* of diabetes if, by the addition of a small amount of peptone, we resuscitate the cells of this organism, suspended in a nutrient medium that contains glucose and ammonia nitrogen, and which, in view of the outcome of the experiment with mannitol, should constitute a complete medium? And in studying the beneficial but as yet rather mysterious insulin effect, is there not reason to pay attention to the possibility that under the influence of this hormone chemically readily detectable substances may be excreted into the blood stream whose presence suffices to

induce the oxidative degradation of the sugar by the body cells?* I gladly leave the answer to these questions to those more competent; but I believe that it was not unwarranted to pose them here, particularly because there is an ever-increasing amount of evidence in favour of a fundamental unity in the mechanism of biological oxidations that extends to everything that lives aerobically; a final example may suffice to illustrate this.

It has long been known that rancid coconut oil contains, among other substances, various ketones, such as methyl nonyl ketone, methyl heptyl ketone, etc. Dr. Derx has recently called my attention to investigations by Stokoe [1922] and by himself, demonstrating that these substances originate under the influence of moulds which apparently produce them by an incomplete oxidation of the fatty acids formed from the oil. Now it is certainly most striking that these microbes effect an incomplete oxidation of, *e.g.*, lauric acid with the formation of methyl nonyl ketone, in complete agreement with Knoop's theory of fatty acid oxidation by higher organisms, according to which the biological oxidation of fatty acids is initiated at the β -carbon atom, thereby producing β -keto acids from which ketones are formed by decarboxylation, a type of conversion that, in the body of the diabetic, causes the production of compounds such as acetoacetic acid and acetone. And many other arguments could be advanced in support of the thesis that the mechanism of oxidative dissimilatory processes reveals a high degree of unity.

It seems to be a practicable task to determine the successive intermediate oxidation stages of physiologically important compounds such as glucose, glycerol, etc., with numerous micro-organisms that exhibit a low oxidative capacity. At the same time one may attempt to ascertain in a quantitative manner the ease with which the various steps are accomplished. It is hardly doubtful that such investigations will increase our comprehension of the nature of microbial metabolism. But by virtue of the above-mentioned unity it will also be eminently

* Even if one had to assume that the oxidation of glucose proceeds by the roundabout way of glycogen, lactacidogen, and lactic acid – as has been proved for the transformations during muscle contraction by the investigations of Embden, Meyerhof, Laquer, and others – similar considerations would nevertheless apply to the later oxidative phases of this process. See the reviews by F. Laquer, 'Insulin', *Naturwiss.*, 12, 89, 1924, and by O. Meyerhof, 'Die Energieumwandlungen im Muskel', *Naturwiss.*, 12, 181, 1924.

useful for our understanding of the metabolic activities of higher organisms which are much less amenable to experimentation. However this may be, I hope that my discussion may have convinced you that a study of microbial metabolism offers many an intriguing problem to the chemist, particularly in view of both the existing diversity and the manifest unity.

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DIE EINHEIT IN DER BIOCHEMIE

(MIT H. J. L. DONKER)

I. EINLEITUNG

DIE Biochemie hatte sich bis vor kurzem in gewissem Sinne der Hauptsache nach als deskriptive Wissenschaft entwickelt. Die verschiedensten Organismen wurden auf ihre chemischen Leistungen untersucht, und ein ungeheures experimentelles Material hat sich allmählich angehäuft. Wenn man versucht dieses zu überblicken, ist man vor allem erstaunt über die ausserordentliche Verschiedenheit der chemischen Umsetzungen, welche von der lebenden Zelle bewirkt werden können.

Allerdings hat man allmählich gelernt, in den biochemischen Vorgängen zwei Arten zu unterscheiden. Immer deutlicher hat sich gezeigt, dass die grosse Gruppe der hydrolytischen Spaltungen und Veresterungen sich scharf von den anderen Stoffwechselvorgängen abtrennen lässt. In Übereinstimmung hiermit hat C. Oppenheimer in der neulich erschienenen Auflage seines grossen Werkes 'Die Fermente'^{1*} eine Trennung vorgenommen zwischen den die hydrolytischen Vorgänge katalysierenden Enzymen: den Hydrolasen und den übrigen 'eentlichen' Stoffwechselermenten, wofür er gemeinsam mit C. Neuberg² den Klassifikationsnamen 'Desmolasen' in Vorschlag gebracht hat. Oppenheimer bemerkt in diesem Zusammenhange: '... während jene (die hydrolytischen Fermente) nur einfache Spaltungsvorgänge an den sekundären Bindungen des Kohlenstoffes mit O oder N katalysieren, die ohne nennenswerte Abnahme der freien Energie verlaufen, beschleunigen diese Fermente Prozesse, welche die entscheidend wichtigen Kohlenstoffverbindungen voneinander trennen, die "Bindungen lösen", die Desmolyse im Gegensatz zur Hydrolyse bewirken. Wesentlich dabei ist auch der Unterschied, dass diese Vorgänge in ihrer Gesamtheit unter Abgabe von freier Energie verlaufen. Die Desmolasen sind also die eentlichen Stoffwechselermente; sie befördern die entscheidenden Vorgänge, bei denen die Zelle sich die

* Footnotes and references have been assembled in the original form on pp. 262—267.

chemische Energie der ihr zugeführten Nährstoffe oder auch der eigenen Leibessubstanz nutzbar macht, sie in andere Energieformen, vor allem in Wärme und mechanische Leistung umsetzt. Für diese Vorgänge sind die Wirkungen der Hydrolasen nur die allerdings unumgängliche Vorbereitung'.³

Wir zitieren hier diese Ausführungen Oppenheimers nur, um von vornherein klarzustellen, dass man zweifellos berechtigt ist, die hydrolytischen Vorgänge von den eigentlichen Stoffwechselprozessen abzutrennen. In dieser Abhandlung werden nämlich die biochemischen Hydrolysen grösstenteils unberücksichtigt bleiben; die Aufmerksamkeit soll nur auf die 'eigentlichen' Stoffwechselprozesse gerichtet werden.

Wenn man also die biochemischen Hydrolysen ausser Betracht lässt, wird man jedoch vom physiologischen Standpunkte aus in den restierenden Stoffwechselvorgängen immer noch zwei Arten unterscheiden können, nämlich die sogenannten dissimilatorischen und die assimilatorischen Prozesse.⁴ Sogar eine oberflächliche Betrachtung des Gesamtstoffwechsels lehrt, dass ein Teil der Nahrung in Produkte übergeführt wird, welche von der Zelle entweder sofort oder doch bald ausgeschieden werden (Dissimilation), während ein anderer Teil der Nahrung als mehr oder weniger wichtige Zellsubstanz zurückgehalten wird (Assimilation). Beide Prozesse sind jedoch aufs innigste miteinander verknüpft, wie wir im 6. Teil näher auseinandersetzen werden.

Es sind die dissimilatorischen Stoffwechselvorgänge, die gewöhnlich mit den Begriffen 'Atmung' und 'Gärung' angedeutet werden, welche wir an erster Stelle näher betrachten wollen. Dann werden wir auch den assimilatorischen Vorgängen unsere Aufmerksamkeit widmen.

Weil es erwünscht ist, den gemeinsamen Charakter von Atmung und Gärung fortwährend klar vor Augen zu haben, haben wir es für angebracht gehalten, die Begriffe 'oxydative Dissimilation' und 'fermentative Dissimilation' einzuführen. Wir sind uns bewusst, dass wir uns damit in Gegensatz stellen zu der üblichen Nomenklatur. Bedauerlicherweise werden auch in neueren Arbeiten typische aerobe Atmungsprozesse, die also nur unter Aufnahme des freien Sauerstoffs vor sich gehen, noch öfters mit dem Namen Gärung ('Fermentation' in französischer und englischer Sprache) belegt.⁵ Wie unerwünscht die-

ser Verwirrung stiftende Brauch ist, folgt ohne weiteres aus der Tatsache, dass die Stoffwechselforschung in neuester Zeit allgemein wieder die Richtigkeit des Kerns des alten Pasteurschen Satzes 'La fermentation est la vie sans air' anerkennt.⁶ Man sollte daher die Begriffe 'Gärung', 'fermentation', 'fermentativ' reservieren für diejenigen Dissimilationsprozesse, welche im Gegensatz zu den Atmungsprozessen ohne Mitwirkung des freien Sauerstoffs vor sich gehen.⁷

Während nun die Gleichwertigkeit von Atmung und Gärung in physiologischer Hinsicht schon zur allgemeinen Anerkennung gelangt ist, hat man in den allerletzten Jahren auch angefangen darauf hinzuweisen, dass diesen, beim ersten Blick in rein chemischer Hinsicht so verschiedenen Prozessen, doch ebenfalls eine weitgehende chemisch übereinstimmende Grundlage eigen ist.

Seit langem hat man schon ausser Zweifel gestellt, dass der Sauerstoff in den weitaus meisten Fällen bei den aeroben Atmungsprozessen erst in das Spiel der Umsetzungen eingreift, nachdem das Atmungssubstrat eine anaerobe Umsetzung durchgemacht hat. Mit dieser Erkenntnis war der Gegensatz im Chemismus der Atmungs- und Gärungsprozesse schon in bedeutender Weise aufgehoben. Immerhin blieb dieser Gegensatz noch in auffallender Weise bestehen, weil die letzte Phase des Atmungsprozesses als Verbrennungsreaktion einen grossen Unterschied im Vergleich mit den Teilprozessen des Gärungsvorganges aufzuweisen schien.

In neuester Zeit ist aber auch diese Lücke in der einheitlichen Betrachtung des Chemismus von Atmung und Gärung auf glücklichste Weise beseitigt worden.

Als wichtigster Beitrag in dieser Hinsicht ist zweifellos die von Heinrich Wieland entwickelte Theorie des Chemismus der aeroben Atmungsprozesse zu betrachten.⁸ Während es sich erübrigt an dieser Stelle darauf näher einzugehen, möge nur kurz angedeutet werden, dass durch Wieland ein überwältigendes Material zusammengebracht wurde zur Erhärtung seiner Auffassung – für welche Palladin⁹, Bredig¹⁰ u.a. auch schon früher, jedoch mit wenigerem Nachdruck, eingetreten waren –, dass das Wesen der biochemischen Oxydationskatalyse an erster Stelle auf eine Aktivierung von Wasserstoffatomen im Oxydationssubstrat (eventuell nach vorhergehender Hydratierung) zurückzuführen sei, während die Rolle des Sauerstoffs zurückgedrängt wurde, indem derselbe nur als Akzeptor dieses aktivierten Wasserstoffs Be-

deutung habe. Diese letzte Ansicht wurde dadurch plausibel gemacht, dass auch bei vielen biologischen Oxydationen der Sauerstoff durch andere gut reduzierbare Substanzen, also durch andere 'Wasserstoffakzeptoren', ersetzt werden konnte.

Die Wielandsche Theorie des Chemismus der aeroben Atmungsprozesse hat schwer um Anerkennung ringen müssen. Dies ist dadurch zu erklären, dass sie in einem Zeitpunkt zur vollen Entwicklung gekommen war, als die Ergebnisse der bewundernswerten Untersuchungen von O. Warburg über die Rolle des Eisens bei der biochemischen Oxydationskatalyse die Aufmerksamkeit der Biochemiker auf sich lenkten.¹¹ Wenn auch auf die Warburgschen Auffassungen hier nicht näher eingegangen werden wird, wollen wir hier nur feststellen, dass unabhängig von Fleisch, von v. Szent-Györgyi und auch von uns darauf hingewiesen wurde, welche grossen Vorteile eine Vereinigung der Wielandschen und der Warburgschen Ansichten über den Chemismus der Atmungsvorgänge bietet. Dafür braucht man nur anzunehmen, dass der gasförmige Sauerstoff an sich ungeeignet ist, als Akzeptor des nach Wieland aktivierten Wasserstoffes zu dienen, und dass der Sauerstoff – im Gegensatz zu den andern Wasserstoffakzeptoren – in Übereinstimmung mit den Ansichten Warburgs sich nur mit dem aktivierten Wasserstoff vereinigen kann, wenn er vorher von einer in der Zelle anwesenden Eisenkomponente in eine höherwertige Eisen-Sauerstoffverbindung übergeführt worden ist.

Auch Oppenheimer hat sich neulich in einer mustergültigen kritischen Betrachtung des vorhandenen experimentellen Materials zugunsten dieser Auffassung ausgesprochen.

Das wichtigste Ergebnis dieser 'einheitlichen Deutung' – wie Oppenheimer sie nennt – des Chemismus der Atmungsprozesse ist nun zweifellos, dass sie die Oxydationsvorgänge in engsten Zusammenhang bringt mit bestimmten Teilprozessen der Gärungsvorgänge. Diese hat man allmählich immer mehr auf Teilreaktionen zurückgeführt, bei denen bestimmten Zwischenprodukten der Gärung Wasserstoff entzogen wurde, welcher alsdann auf ein anderes Zwischenprodukt übertragen wurde. Das bekannteste, von Neuberg immer wieder in den Vordergrund gerückte Beispiel, ist die Cannizzaro-Umlagerung oder 'Dismutation' der Aldehyde, bei der die eine Hälfte der Aldehydmenge zu Säure dehydriert, die andere Hälfte zu Alkohol reduziert wird. Doch auch auf andere gekuppelte Dehydrierungs- und

Hydrierungsvorgänge im anaeroben Abbau der Dissimilationssubstrate ist schon mehrmals die Aufmerksamkeit gelenkt worden.

Wir können aus den obigen Betrachtungen schliessen, dass *die grosse Bedeutung der Oxydoreduktion* – wie wir die gekuppelte Dehydrierung und Hydrierung kurz bezeichnen können – für die Erklärung des Chemismus der Dissimilationsvorgänge immer deutlicher in den Vordergrund tritt.

Die obenstehenden Ausführungen beabsichtigen nur eine kurze Übersicht von dem heutigen Stande unserer Kenntnisse des Chemismus der Dissimilationsvorgänge zu geben. Für die Dokumentierung dieser Betrachtungsweise kann in erster Linie auf die schon erwähnte rezente Zusammenfassung von Oppenheimer verwiesen werden.

Das an jener Stelle zusammengebrachte Material beschränkt sich jedoch der Hauptsache nach auf die aeroben Atmungsprozesse; von den Gärungsprozessen wird fast nur die alkoholische Gärung berücksichtigt. Das der mikrobiologischen Stoffwechselforschung entnommene Material, das uns in unserer vorläufigen Mitteilung selbständig zu ähnlichen Schlüssen geführt hat, ist ausser Betracht geblieben, obwohl unsere diesbezügliche Abhandlung beiläufig erwähnt worden ist.¹²

Wir glauben daher, dass es nützlich sein wird, unsere Betrachtungen hier kurz wiederzugeben, um so mehr als wir dabei zu Vorstellungen und Schlüssen gelangt sind, die nach unserer Meinung von grösserer Tragweite sind.

Während nämlich Oppenheimer in Übereinstimmung mit der üblichen Auffassung zwar den Oxydoreduktionsvorgängen eine hervorragende Stellung bei den Dissimilationsprozessen zuschreibt, bemerkt er doch ausdrücklich: 'Andererseits umfasst aber der Begriff Oxydoreduktasen auch wieder nicht das gesammte, chemisch und biologisch zusammengehörige Gebiet, da andere wichtige Teilfermente nicht dazugehören, wie die Karboxylase u.a.'¹³

Dagegen haben wir schon vor einem Jahre darauf hingewiesen, dass *alle* Teilprozesse der Dissimilation – soweit nicht Veresterungen und Hydrolysen eingreifen¹⁴ – als Oxydoreduktionsprozesse zu betrachten sind.

Wenn auch diese Aussprache an und für sich bedeutungslos scheinen kann, so befähigt sie doch zu Schlüssen, die geeignet sind, in hohem Grade Ordnung in das Chaos der biochemischen Erscheinungen zu bringen. Dies möge in einigen Zügen angedeutet werden.

Angesichts der Tatsache, dass man in sehr vielen Fällen mit gutem

Erfolge versucht hat, die von der lebenden Zelle hervorgerufenen biochemischen Umsetzungen auch mit den aus der Zelle abgetrennten Enzymen zu bewirken, hat sich die Vorstellung allgemein eingebürgert, dass eine Zelle ein Magazin ist, das mit den verschiedensten Werkzeugen gefüllt ist. Jedes dieser Werkzeuge befähigt die Zelle zu einer speziellen chemischen Reaktion. In welcher Weise diese Werkzeuge über die verschiedenen Zellen verteilt sind, entzieht sich dabei jeder theoretischen Überlegung. Es scheint, dass jede artspezifische Zelle durch das Vorkommen einer bestimmten, aber willkürlichen, Enzymkombination gekennzeichnet ist.

Wie weit diese Auffassung, wenn man auch die hydrolytischen Enzyme in Betracht zieht, berechtigt ist, werden wir hier dahingestellt lassen. Beschränken wir uns aber auf die eigentlichen Dissimilationsvorgänge, dann folgt aus dem Zurückführen aller Teilprozesse auf Oxydoreduktionen die Möglichkeit, den ganzen Vorgang der Dissimilation für eine Zelle einem und demselben Agens zuzuschreiben. Die Unterschiede, welche die Dissimilationsvorgänge der verschiedenartigen Zellen aufweisen, sollten bei dieser Auffassung ihre Ursache finden in einer quantitativen Abstufung einer und derselben Eigenschaft des oxydoreduzierenden Agens. Indem wir nun diese Eigenschaft auf Grund der neueren Auffassungen über die chemische Katalyse näher zu präzisieren versuchten, ergab sich die Möglichkeit, dabei einen Zusammenhang mit ganz andern Eigenschaften der lebenden Zellen durchaus plausibel zu machen.

Wir hoffen dies durch die folgenden Ausführungen näher zu erläutern und den Wert unserer Betrachtungsweise wenigstens als Arbeitshypothese begründen zu können.

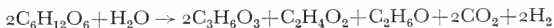
2. DIE ZURÜCKFÜHRUNG DER DISSIMILATIONSPROZESSE AUF KATALYTISCHE WASSERSTOFFÜBERTRAGUNG, FALLS ZUCKER ALS SUBSTRATE FUNGIERTEN

Die Veranlassung zu unseren Betrachtungen war der Wunsch, die äusserst verschiedenen Umsetzungen, welchen Zuckerarten bei den fermentativen Dissimilationsvorgängen der unterschiedenen Mikrobenarten unterliegen, von einem gemeinschaftlichen Gesichtspunkte aus zu überblicken. In unserer ersten, oben zitierten Mitteilung haben wir näher begründet, wie es möglich ist, die zur fermentativen Zucker-

dissimilation befähigten Mikrobenarten in einer sehr beschränkten Zahl natürlicher Gruppen unterzubringen.

Die Vernachlässigung dieses Gesichtspunktes ist einer der Gründe, weshalb unsere Kenntnis über die Art des mikrobiellen Zuckerabbaus bis heute noch so mangelhaft ist.

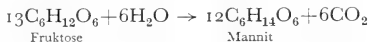
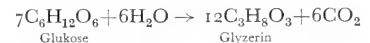
Ein zweiter Grund für diese Situation liegt darin, dass man bis vor kurzem wenig Erfolg mit den Versuchen gehabt hat, sich eine nähere Vorstellung von dem Chemismus der für die verschiedenen Gruppen charakteristischen Art des Zuckerabbaus zu machen. Anfänglich hat man versucht, die von einem Organismus bewirkte Umsetzung durch eine einzelne chemische Gleichung wiederzugeben. Als Beispiel sei nur die vor langer Zeit von Harden¹⁵ für die Glukosegärung durch *Bacterium coli* vorgeschlagene Gleichung angeführt:



Eine derartig einfache zusammenfassende Formel kann jedoch nicht befriedigen, schon deshalb nicht, weil die Quantitäten der entstandenen Gärungsprodukte je nach den äusseren Umständen in hohem Grade wechseln.

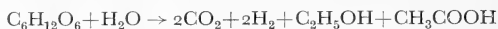
Dieser Umstand hat einige Forscher dazu gebracht, eine Erklärungsweise zu geben, wobei angenommen wurde, dass jedes der gefundenen Gärungsprodukte sein Entstehen einer besonderen, unabhängig vor sich gehenden Umbildung des Zuckers verdankt.¹⁶ In den Fällen, bei denen während der Gärung Kohlensäure und Wasserstoff gebildet wird, ist es selbstverständlich leicht, für jedes Gärungsprodukt eine einfache Gleichung niederzuschreiben, wobei dieses Produkt unter Freiwerden der beiden genannten Gase aus dem Zuckermolekül hergeleitet wird.

Dagegen springt sofort ins Auge, zu welchen unhaltbaren Konsequenzen eine derartige Auffassung für diejenigen Gärungsprozesse führt, bei welchen kein freier Wasserstoff entsteht. So nimmt z.B. Baumgärtel¹⁷, um auf diese Weise das Entstehen von Produkten wie Glycerin bzw. Mannit bei der Milchsäuregärung zu erklären, seine Zuflucht zu Gleichungen wie:



Zwischen diesen beiden Extremen steht die Auffassungsweise, nach welcher bei den meisten Gärungsprozessen eine Anzahl mehr oder weniger unabhängiger Umsetzungen eintreten. Die gebildeten Gärungsprodukte werden unter dieser Voraussetzung teilweise gleich gerichteten, teilweise verschieden gerichteten Umsetzungen ihr Entstehen verdanken.

Eines der wenigen ausgearbeiteten Beispiele hierfür findet man im Studium von Grey¹⁸ über die Glukosevergärung durch *Bacterium coli*. Hierbei wird die Bildung der Milchsäure unabhängig von der Entstehung der übrigen Produkte gedacht, die aus dem Glukosemolekül gemäss folgender Gleichung entstehen:



Obleich diese letzte Auffassung zweifellos die richtige ist, hat man sie bis jetzt noch wenig zur Erklärung des Chemismus der verschiedenen Gärungsprozesse benützt.

Eine neue Epoche im Studium der Gärungsprozesse wurde eingeleitet von den grundlegenden Untersuchungen von Neuberg¹⁹ und seinen Mitarbeitern über die alkoholische Gärung. Bei diesen Untersuchungen wurde u.m. der Nachweis erbracht, dass der bei genannter Gärung auftretende Alkohol in letzter Instanz durch Reduktion von Azetaldehyd gebildet wurde.²⁰ Später folgte eine Reihe von Untersuchungen über verschiedene, durch andere Mikroben bewirkte fermentative Zuckerdissimilationsprozesse, woraus sich ergab, dass aller Wahrscheinlichkeit nach auch hierbei Azetaldehyd als Zwischenprodukt auftritt.

Ausser bei der alkoholischen Gärung, wiesen sie dies nach für die Zuckervergärung durch *B. coli communis*, *B. lactis aerogenes*, *B. dysenteriae*, 'Gasbrandbazillus', *Bac. butylicus* Fitz und *Bac. butyricus* Fitz, wobei sie ebenfalls Azetaldehyd mit Hilfe von Natrium- oder Kalziumsulfid bzw. Dimedon 'abfangen' konnten.²¹

Auch Peterson und Fred konnten auf dieselbe Weise Azetaldehydbildung für ein dem *B. coli* nahestehendes Bakterium, für die sporenbildende Art *Bac. acetoethylicum* und für ein Milchsäurebakterium, nämlich *Lactobacillus pentoaceticus* nachweisen.²² Durch De Graaff und Le Fèvre wurde dieser Nachweis auch für weitere Vertreter der Koli-Typhusgruppe (*B. typhosum*, *B. paratyphosum* A und B) erbracht.²³

Auf diese Beobachtungen – welche noch durch viele andere, auf die

hier nicht eingegangen werden kann, gestützt werden – gründet Neuberg die überaus wichtige Ansicht, dass bei den verschiedenen Zuckergärungen zunächst überall die gleichen Spaltungen auftreten, und dass die beobachteten Unterschiede in den Gärungsendprodukten nur auf Unterschiede in den *sekundären* Umwandlungen der primären Spaltungsprodukte zurückzuführen sind.

Obgleich diese Erkenntnis zweifellos einen gewaltigen Fortschritt bedeutet, genügt sie nicht, um ohne weiteres eine klare Einsicht in das Spiel der Umsetzungen zu geben und die Entstehung der sehr zahlreichen Gärungsprodukte zu erklären. Während bei der alkoholischen Gärung, bei der die Produkte des Zuckerabbaus sich praktisch auf Äthylalkohol, Kohlensäure und ein wenig Glycerin beschränken, ein wichtiger Teil des Vorganges klargelegt worden ist, waren die Umsetzungen des Zuckermoleküls bei den bakteriellen Gärungen noch grösstenteils im Dunkeln geblieben.

Im folgenden wollen wir auseinandersetzen, wie die Vorgänge in allen Fällen *vollständig* auf eine Kette von Oxydoreduktionen, oder wie wir es lieber ausdrücken möchten, auf eine katalytische Übertragung von Wasserstoff zurückzuführen sind.

Während wir uns in diesem Teil bis jetzt auf eine Betrachtung der fermentativen Zuckerdissimilationsprozesse beschränkt haben, wollen wir gemäss der Einleitung jetzt auch die oxydativen Zuckerdissimilationsprozesse in Betracht ziehen. Denn hierdurch wird deutlich werden, wie die Wielandsche Auffassung der aeroben Atmungsprozesse als Dehydrierungsvorgänge einen überaus glücklichen Anschluss dieser Prozesse an die Gärungsprozesse ermöglicht und eben diese Tatsache ein wichtiges Argument zugunsten dieser Auffassung liefert.

Wie gesagt, werden wir uns an dieser Stelle auf die Betrachtung eines einzelnen Dissimilationssubstrates, nämlich der Glukose, beschränken.²⁴ Dabei wird klar ans Licht kommen, dass, wie verschiedenartig die Umsetzungen in den verschiedenen Organismengruppen auch sein mögen, sie alle vollständig auf Oxydoreduktionswirkungen zurückzuführen sind.

Wir sind uns dabei bewusst, dass eine vollständige Dokumentierung der von uns in den einzelnen Fällen angenommenen Reaktionen des Zuckerabbaus nicht gegeben wird. Doch sprechen dafür in den meisten Fällen wichtige Argumente, auf die wir aber leider mit Hinsicht auf den Rahmen dieser Abhandlung nicht ausführlich eingehen können.

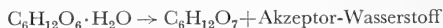
Wir werden unsere Ausführungen mit einer Betrachtung der Organismen mit ausgesprochen oxydativem Charakter anfangen und allmählich immer mehr fermentativ geneigte Organismen folgen lassen, um schliesslich mit den völlig anaeroben Organismen zu enden.

I. Organismen mit ausgesprochen oxydativem Charakter

Beispiele hierfür sind die Essigsäurebakterien und zahlreiche Schimmelpilze.

Wie längst bekannt, wird Glukose von allen Essigsäurebakterien und zahlreichen Schimmelpilzen an erster Stelle zu Glukonsäure oxydiert. Nach der Wielandschen Auffassung beruht dieser Vorgang auf einer Dehydrierung des Glukosemoleküls, wobei aber vorher an der Aldehydgruppe eine Hydratierung stattgefunden hat.

Diese Dehydrierung findet aber in diesen Fällen nur statt, wenn gleichzeitig ein kräftiger Wasserstoffakzeptor anwesend ist, also:



Normalerweise ist dieser Akzeptor der – gemäss der Warburgschen Ausführungen zunächst an organisch gebundenem Eisen aktivierte – Sauerstoff, welcher dabei schliesslich in Wasser übergeführt wird. Doch konnte Wieland zeigen, dass für die Oxydation von Äthylalkohol der Sauerstoff auch von andern kräftigen Akzeptoren wie Methylblau ersetzt werden kann. Wir haben diesen Befund auch für die Glukoseoxydation feststellen können.

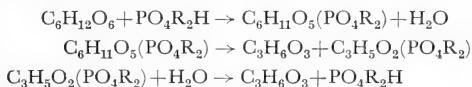
Die gebildete Glukonsäure unterliegt dann unter dazu günstigen Bedingungen einer ganzen Reihe weiterer Dehydrierungen, ebenfalls mit Sauerstoff als Akzeptor, wobei in vielen Fällen wahrscheinlich Zitronensäure, Fumarsäure und Oxalsäure als Zwischenprodukte auftreten, welche schliesslich zu den Endprodukten Kohlensäure und Wasser führen.²⁵

Bei allen weiter unten zu besprechenden Umsetzungen findet eine direkte Dehydrierung des Glukosemoleküls nicht statt. Zahlreiche Belege sind dafür vorhanden, dass der Zucker in den weitaus meisten Fällen zuerst in Verbindungen vom C_3 -Typus übergeführt wird. Über die Art der dabei auftretenden Reaktionen sind wir noch ungenügend

unterrichtet. Doch ist die Hypothese, dass diese Umsetzung in allen Fällen über den Glycerinaldehyd zu dem Methylglyoxal(hydrat) führt, wie Neuberg dies für die alkoholische Gärung der Zucker angenommen hat, durchaus wahrscheinlich. Dabei ist aber zu berücksichtigen, dass bei dieser Spaltung des Glukosemoleküls die von Harden und Young²⁶ bei der alkoholischen Gärung entdeckte biochemische Phosphorylierung eine ausschlaggebende Rolle spielt.

Angesichts der Tatsache, dass eine ähnliche Phosphorylierung des Zuckers von Embden und Laquer²⁷ auch bei dem Kohlenhydratstoffwechsel im Muskelgewebe festgestellt worden ist und Virtanen sie auch für die Zuckerdissimilation durch wahre Milchsäurebakterien²⁸ und durch Propionsäurebakterien²⁹ ausser Zweifel gestellt hat, ist es sehr wahrscheinlich, dass die Zuckerspaltung immer unter Beteiligung der anorganischen Phosphate vor sich gehen wird.³⁰

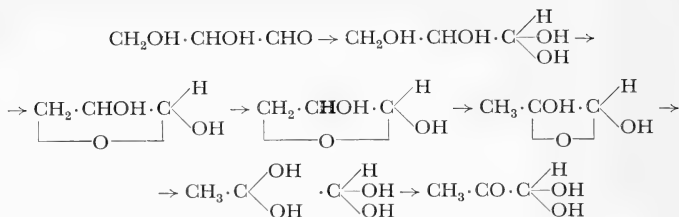
In einer neulich erschienenen Abhandlung hat einer von uns (Kl.) gemeinsam mit A. P. Struyk³¹ dargetan, dass viele wichtige Argumente dafür sprechen, dass der Verlauf der primären Zuckerspaltung wie folgt vor sich geht:



In diesen Gleichungen steht für den Fall, dass Glukose gespalten wird, $\text{C}_3\text{H}_6\text{O}_3$ für Glycerinaldehyd. Wie eine nähere Betrachtung des Chemismus des eigentlichen Spaltungsvorganges des Glukose-monophosphorsäureesters uns gelehrt hat, ist dieser Vorgang zurückzuführen auf eine intramolekulare Dehydrierung und Hydrierung, wobei das an das vierte C-Atom gebundene Wasserstoffatom auf das dritte C-Atom übertragen wird, unter gleichzeitiger Sprengung der Bindung zwischen diesen beiden C-Atomen.

Der gebildete Glycerinaldehyd unterliegt nun weiteren Umlagerungen, welche ebenfalls auf eine intramolekulare Dehydrierung und Hydrierung zurückzuführen sind, und wobei schliesslich Methylglyoxalhydrat resultiert.

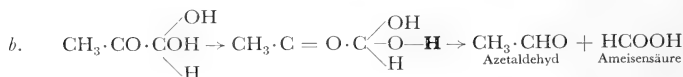
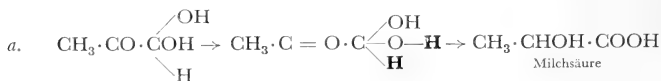
Die wahrscheinlichste Vorstellung dieser Umlagerung ist wohl die hierunter folgende:

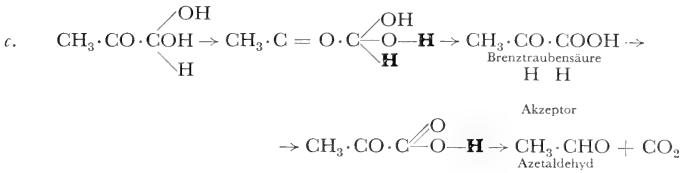


In diesem Schema ist das vom wirksamen Agens aktivierte Wasserstoffatom als **H** angegeben, wie dies auch in den weiteren Ausführungen immer geschehen wird. Für die weitere Erklärung wird nur Gebrauch gemacht von der durchaus berechtigten Annahme, dass die genannten Verbindungen alle Neigung haben, in wässriger Lösung Gleichgewichte zu bilden mit isomeren Verbindungen, welche durch Hydratierung, Ringschluss (Wasserabspaltung) und Ringöffnung (ebenfalls Hydratierung) aus ihnen entstehen. Das entscheidende Moment dabei ist nur die vom wirksamen Agens katalysierte Übertragung des Wasserstoffatoms vom zweiten auf das dritte C-Atom.

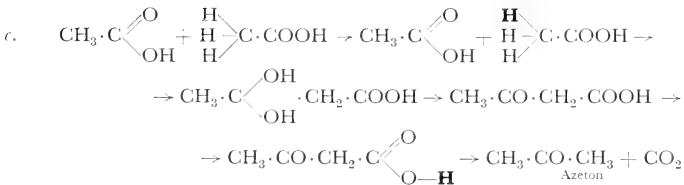
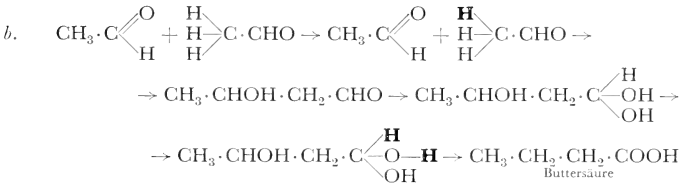
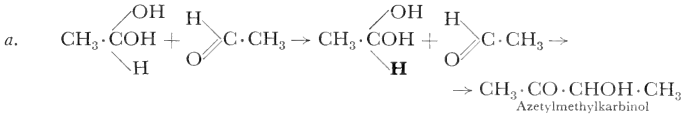
Wir sind nun der Ansicht, dass alle bei den weiter zu besprechenden Arten des Zuckerabbaus angetroffenen Verbindungen aus dem Methylglyoxalhydrat durch eine Reihe von Oxydoreduktionen gebildet werden. Um bei der Behandlung der einzelnen Organismengruppen Wiederholungen zu vermeiden, haben wir alle in Betracht kommenden Reaktionen in das folgende Schema untergebracht. Dabei sind die vom wirksamen Agens im Sinne Wielands aktivierten Wasserstoffatome durch Fettdruck hervorgehoben worden. Die betreffenden Reaktionen sind je nach ihrem Charakter in Gruppen vereinigt, welche wohl keiner Erläuterung bedürfen.

I. Umlagerungen des Methylglyoxalhydrats

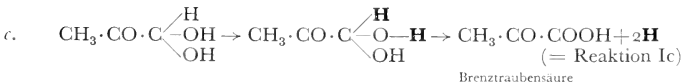
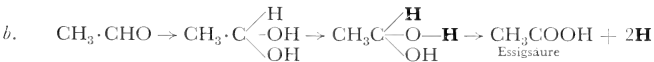




II. Kondensationsreaktionen



III. Dehydrierungsreaktionen



IV. Hydrierungsreaktionen

- a. $2\mathbf{H} \rightarrow \text{H}_2$
gasförmiger Wasserstoff
- b. $\text{O} + 2\mathbf{H} \rightarrow \text{H}_2\text{O}$
aktivierter Sauerstoff
- c. $\text{CH}_2\text{OH} \cdot \text{CHOH} \cdot \text{CHO} + 2\mathbf{H} \rightarrow \text{CH}_2\text{OH} \cdot \text{CHOH} \cdot \text{CH}_2\text{OH}$
Glycerin
- d. $\text{CH}_3 \cdot \text{CHOH} \cdot \text{COOH} + 2\mathbf{H} \rightarrow \text{CH}_3 \cdot \text{CH}_2 \cdot \text{COOH}$
Propionsäure
- e. $\text{CH}_3 \cdot \text{CHO} + 2\mathbf{H} \rightarrow \text{CH}_3 \cdot \text{CH}_2\text{OH}$
Äthylalkohol
- f. $\text{CH}_3 \cdot \text{CO} \cdot \text{CHOH} \cdot \text{CH}_3 + 2\mathbf{H} \rightarrow \text{CH}_3 \cdot \text{CHOH} \cdot \text{CHOH} \cdot \text{CH}_3$
2,3-Butylenglykol
- g. $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH} + 4\mathbf{H} \rightarrow \text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2\text{OH} + \text{H}_2\text{O}$
n-Butylalkohol
- h. $\text{C}_6\text{H}_{12}\text{O}_6 + 2\mathbf{H} \rightarrow \text{C}_6\text{H}_{14}\text{O}_6$
Fructose Mannit
- i. usw.

Hierzu ist nur noch zu bemerken, dass die Dehydrierungsreaktionen immer gekoppelt mit der einen oder anderen Hydrierungsreaktion verlaufen, wenn man jedenfalls – wie wir es getan haben – die eventuell stattfindende Entwicklung gasförmigen Wasserstoffs auch als eine Hydrierung (des atomaren Wasserstoffs) betrachten will.

Wir wollen nun kurz darauf hinweisen, dass man die in den jetzt folgenden Gruppen auftretenden Formen des Zuckerabbaues nahezu vollständig auf bestimmte Kombinationen der im Schema aufgenommenen Reaktionen zurückführen kann.

II. Organismen mit obligat aerobem Charakter, aber mit etwas geringerem Oxydationsvermögen als die Gruppe I

Hierzu gehören die Zellen der höheren Pflanzen und Tiere (wenigstens die des Muskelgewebes), wie auch viele aeroben Bakterien.

Beim Muskelgewebe der höheren Tiere wird das gebildete Methylglyoxalhydrat unter anaeroben Bedingungen in Milchsäure umgelagert (Reaktion Ia). Diese Säure wird dann unter aeroben Bedingungen dehydriert, wobei der Sauerstoff als Akzeptor auftritt (Reaktion IVb). Normalerweise findet aller Wahrscheinlichkeit nach bei der Ruheatmung schon vor dem Auftreten der Milchsäure eine Dehydrierung von Methylglyoxalhydrat mit Sauerstoff als Akzeptor statt, wobei Brenztraubensäure entsteht, welche Säure dann einer intramole-

kularen Dehydrierung und Hydrierung zu Azetaldehyd und Kohlensäure unterliegt (Reaktion *Ic*). Der Azetaldehyd wird dann weiter dehydriert bis zu Kohlensäure und Wasser, wobei ebenfalls Sauerstoff als Akzeptor auftritt (Reaktion *IVb*).

Bei den höheren Pflanzen findet unter anaeroben Bedingungen eine Umlagerung statt, wie sie bei den Alkoholhefen angetroffen wird (vergleiche die nächstfolgende Gruppe). Unter normalen Bedingungen findet man dieselben Verhältnisse wie für die Ruheatmung des Muskelgewebes angegeben. Bemerkt sei nur noch, dass in dieser Gruppe die unter anaeroben Bedingungen verlaufenden Umsetzungen nicht genügen, um das Energiebedürfnis der Zelle zu befriedigen.

Insofern die Organismen der nachfolgenden Gruppen III, IV und V in der Lage sind, den Zucker unter Sauerstoffzutritt zu dissimilieren, wird der (aktivierte) Sauerstoff in die Gärungsvorgänge eingreifen, indem er an die Stelle der Gärungsakzeptoren tritt. Gerade wie bei der Gruppe II wird dieser Eingriff in letzter Instanz zu der Bildung von Kohlensäure und Wasser führen.

In den folgenden Abschnitten wird jedoch nur die fermentative Zuckerdissimilation betrachtet werden.

III. Organismen, welche zur alkoholischen Vergärung des Zuckers befähigt sind

Hierzu gehören einzelne Schimmelpilze wie *Mucor racemosus* und vor allem die Alkoholhefen.

Der Zuckerabbau verläuft hier gemäss der bekannten Anschauung Neubergs mittels der Reaktionen *Ic* und *IVe*. Da aber der für die erste Phase der Reaktion *Ic* benötigte Akzeptor erst bei der zweiten Phase dieser Reaktion gebildet wird, ist es klar, dass die alkoholische Gärung einen einleitenden Akzeptor benötigt. Unter Umständen wird vielleicht der Glyzerinaldehyd diese Rolle spielen und dabei nach Reaktion *IVc* in Glyzerin übergeführt werden. Es ist aber nicht unwahrscheinlich, dass in vielen Fällen der freie Sauerstoff der Luft seine Akzeptorwirkung entfalten wird (Reaktion *IVb*).

IV. Die fakultativ anaeroben zur fermentativen Zuckerdissimilation befähigten sporenbildenden Bakterien

Man kann in dieser Gruppe zwei Untergruppen unterscheiden:

a. Die Untergruppe von *Bac. acetoethylicum* Northrop

Hierzu gehört auch die bekannte Art *Bac. macerans* Schardinger. Der fermentative Zuckerabbau ist hier eingehend von verschiedenen amerikanischen Forschern studiert. Die Gärungsprodukte waren: Äthylalkohol, Azeton, Wasserstoff, Kohlensäure, Essigsäure, Ameisensäure und Milchsäure.³²

Die sich hier abspielenden Reaktionen sind vor allem die Reaktionen *Ib* und in geringem Masse auch *Ia*. Die Gärungsprodukte Äthylalkohol, Essigsäure und Azeton entstehen zweifellos durch die Reaktionen *IVe*, *IIIb* und *IIIb* + *IIc* aus dem Azetaldehyd.

b. Die Untergruppe von *Bac. polymyxa* Beijerinck

Hierzu gehört auch *Bac. asteroides* Arthur Meyer. Diese Untergruppe ist eingehend von dem einen von uns (D.) untersucht.³³

Hier treten 2,3-Butylenglykol, Äthylalkohol, Kohlensäure und Wasserstoff als Hauptprodukte auf. Auch hier entstehen der Azetaldehyd und die Kohlensäure hauptsächlich nach Reaktion *Ib* und *IIIa*. Das 2,3-Butylenglykol entsteht zweifellos nach den Reaktionen *IIa* und *IVf*.

V. Die Bakterien der Koli-Typhus-Gruppe

Die Vorgänge bei der fermentativen Zuckerdisimilation der Bakterien dieser Gruppe sind vor allem von Harden³⁴ und von Grey³⁵ aufgeklärt worden. Weitere Angaben sind bei Neuberg und Nord und bei De Graaff und Le Fèvre anzutreffen.

Es empfiehlt sich, hier drei Untergruppen zu unterscheiden:

a. Untergruppe von *B. typhosum*

Die ersten Reaktionen sind zweifellos *Ia* und *Ib*. Der bei der letzten Reaktion gebildete Azetaldehyd wird gemäss den Reaktionen *IIIb* und *IVe* in Essigsäure und Äthylalkohol übergeführt.

b. Untergruppe von *B. coli*

Angeichts der Tatsache, dass Greys 'selected strains' of *B. coli*, bei denen das Vermögen zur Gasbildung verloren gegangen war, einen Stoffwechsel hatten, der grosse Ähnlichkeit mit dem von *B. typhosum* zeigte und somit einen merkbaren Ertrag an Ameisensäure aufwies, ist wohl anzunehmen, dass hier neben Reaktion *Ia* auch Reaktion *Ib* auftritt. Die Kohlensäure und der Wasserstoff werden dabei gemäss Reaktion *IIIa* gebildet. Der Azetaldehyd wird wiederum gemäss den Reaktionen *IIIb* und *IVe* in Essigsäure und Äthylalkohol übergeführt.

Teilweise wird auch Glycerinaldehyd als Akzeptor auftreten und in Glycerin übergeführt (Reaktion IVc). Während wir früher angenommen haben, dass die Bernsteinsäure einer Dehydrierung von Essigsäure entstammte, ist es angesichts der rezenten Untersuchung Virtanens über die Entstehung von Bernsteinsäure bei der Propionsäuregärung (s.d.) und im Zusammenhang mit den quantitativen Ergebnissen Greys wahrscheinlicher, dass die Bernsteinsäure auch hier einer nebenbei vor sich gehenden Spaltung des nicht-phosphorylierten Zuckers in eine C₄- und eine C₂-Verbindung seinen Ursprung verdankt.

c. Untergruppe von *B. aerogenes*

Die Situation ist hier im grossen und ganzen dieselbe wie in Untergruppe b. Nur wird der Azetaldehyd hier nicht ausschliesslich nach den Reaktionen IIIb und IVe umgesetzt, doch gemäss der Reaktion IIa in Azetylmethylkarbinol übergeführt, das dann unter Mitwirkung des aus Reaktion IIIa stammenden Wasserstoffs nach Reaktion IVf in 2,3-Butylenglykol umgewandelt wird.

VI. Die Gruppe der wahren Milchsäurebakterien

Hierbei lassen sich wieder zwei Untergruppen unterscheiden.

a. Die Untergruppe der heterofermentativen Milchsäurebakterien

Der erste, der über die Zuckerdissimilation in dieser Untergruppe eingehende Versuche anstellte, war Jan Smit³⁶, der mit *Lactobacillus fermentum* arbeitete. In neuester Zeit sind ausführliche Untersuchungen mit einer sehr verwandten Bakterienart *Lactobacillus pentoaceticus* gemacht von Fred, Peterson und Mitarbeitern.³⁷

Die Ergebnisse stimmen bei beiden Bakterienarten in der Auffassung überein, dass neben Reaktion Ia auch Reaktion Ib verläuft. Der nach der Reaktion IIIa gebildete Wasserstoff wird dabei quantitativ für die Hydrierungsreaktionen IVc und IVe benutzt.

Wird Fruktose an Stelle von Glukose dissimiliert, dann tritt die Hydrierungsreaktion IVh in den Vordergrund.

b. Die Untergruppe der homofermentativen Milchsäurebakterien

Zu dieser Untergruppe gehören Bakterien wie *Lactobacillus delbrücki*, *Lactobacillus bulgaricus* u.a., welche bekanntlich aus Zucker nur Milchsäure (Reaktion Ia) bilden.

VII. Die Gruppe der Propionsäurebakterien

Über die Zuckerdissimilation in dieser Gruppe sind in den letzten Jahren eingehende Untersuchungen von Virtanen³⁸ veröffentlicht worden. Dieser Forscher fand als Produkte der Zuckervergärung Propionsäure, Essigsäure, Bernsteinsäure und Kohlensäure. Es unterliegt wohl keinem Zweifel, dass das Methylglyoxalhydrat in bedeutender Menge in Milchsäure umgelagert wird gemäss Reaktion Ia. Virtanen hält es nun für wahrscheinlich, dass diese Umlagerung quantitativ verläuft und dass die weiteren Produkte aus Milchsäure entstehen, indem ein Teil dieser Säure unter Kohlensäureabspaltung und gleichzeitiger Oxydation in Essigsäure übergeht. Der dieser Dehydrierung entstammende Wasserstoff würde auf den andern Teil der Milchsäure übertragen werden, wobei diese Säure in Propionsäure übergeführt wird. Es ist jedoch nicht ausgeschlossen, dass ein Teil des Methylglyoxalhydrats gar nicht in Milchsäure, sondern nach unserer Reaktion Ib in Ameisensäure und Azetaldehyd übergeführt wird, welche beide Körper dann dehydriert werden (Reaktionen IIIa und IIIb), wobei ebenfalls Milchsäure als Akzeptor auftritt und in Propionsäure umgesetzt wird (Reaktion IVd). Die gebildete Bernsteinsäure wird aller Wahrscheinlichkeit nach auf anderem Wege, nämlich aus dem nicht phosphorylierten Zucker gebildet.³⁹

VIII. Die Gruppe der Buttersäure- und Butylalkoholbakterien

Über den in den letzten 15 Jahren für die technische Bereitung von Butylalkohol und Azeton herangezogenen Gärungsprozess, wobei Stärke der Hauptsache nach in Butylalkohol, Azeton, Kohlensäure und Wasserstoff umgesetzt wird, sind von englischer, kanadischer und amerikanischer Seite mehrere Publikationen erschienen.⁴⁰ Einer von uns (D.) hat weiter über den Vorgang der Zuckerdissimilation auch für andere Vertreter dieser Gruppe ausgebreitete Versuche angestellt.⁴¹ Wir können hier wieder zwei Untergruppen unterscheiden:

a. Untergruppe der Buttersäurebakterien

In dieser Untergruppe findet zuerst wieder eine Umlagerung des Methylglyoxalhydrats statt in Ameisensäure und Azetaldehyd gemäss unserer Reaktion Ib. Die Ameisensäure wird praktisch vollständig zu Kohlensäure und gasförmigem Wasserstoff dehydriert (Reaktionen IIIa und IVa). Der Azetaldehyd unterliegt dann teilweise einer Um-

lagerung, wobei der Wasserstoff von einem Molekül auf ein zweites, unter gleichzeitiger Kuppelung dieser Moleküle zu Aldol, übertragen wird. Das gebildete Aldol unterliegt weiter einer intramolekularen Dehydrierung und Hydrierung, wobei es in Buttersäure übergeführt wird (Reaktion II*b*). Ein anderer Teil des Azetaldehyds wird in Essigsäure und gasförmigen Wasserstoff umgewandelt (Reaktionen III*b* und IV*a*).

b. Untergruppe der Butylalkoholbakterien

Während in der erstgenannten Untergruppe alle Aufnahme von Wasserstoff durch die anderen Gärprodukte unterbleibt, findet dieselbe in der jetzt zu besprechenden Untergruppe wohl statt. Dabei wird ein Teil des den Dehydrierungen entstammenden Wasserstoffs auf die Buttersäure übertragen, wobei diese in n-Butylalkohol übergeführt wird (Reaktion IV*g*). Ausserdem findet aber eine Übertragung von Wasserstoff von einem Molekül Essigsäure auf ein zweites Molekül, unter gleichzeitiger Kuppelung beider Moleküle zu Azetessigsäure statt, die dann einer intramolekularen Dehydrierung und Hydrierung unterliegt, woraus Azeton und Kohlensäure resultieren (Reaktion II*c*).

Der Unterschied im Verhalten beider Untergruppen wird sehr wahrscheinlich dadurch bedingt, dass die in der zweiten Untergruppe dazugekommenen Reaktionen nur bei einer bestimmten Säurekonzentration mit messbarer Geschwindigkeit vor sich gehen. Die Bakterien der ersten Untergruppe sind nun säureempfindlicher als die Butylalkoholbakterien und sterben schon, bevor der 'kritische' Säuregrad erreicht worden ist.

3. DER CHEMISMUS DER KATALYTISCHEN WASSERSTOFFÜBERTRAGUNG

Wenn wir das in den vorangehenden Teilen Behandelte überblicken, so sehen wir, dass es möglich ist, die verschiedenartigsten dissimilatorischen Umwandlungen des Zuckermoleküls vollständig auf eine Kette katalytischer Übertragungen von Wasserstoff zurückzuführen. Dabei kann diese Übertragung unserer Ansicht nach sowohl *intermolekularer* als auch *intramolekularer* Natur sein. Obwohl die intermolekulare Übertragung als gekoppelter Dehydrierungs- und Hydrierungsvorgang von verschiedenen Forschern mehrfach zur Erklärung bestimmter Stoff-

wechselsvorgänge herangezogen war, stand eine konsequente Anwendung dieses Erklärungsprinzips in der vergleichenden Biochemie noch aus. Ausserdem weicht unsere Anschauung von der anderer Forscher dadurch in prinzipieller Weise ab, dass wir – wie schon in unserer vorläufigen Mitteilung bekannt gegeben – auf die Möglichkeit hingewiesen haben, auch die unter Kohlenstoffverknüpfung vor sich gehenden Kondensationsreaktionen und Umlagerungsreaktionen als eine katalytische Übertragung von Wasserstoff aufzufassen und somit die Möglichkeit zu schaffen, alle Dissimilationsteilprozesse von einem gemeinschaftlichen Gesichtspunkte aus zu überblicken.⁴²

Die Berechtigung dieser Anschauung wird am deutlichsten hervortreten, wenn wir jetzt den Chemismus der katalytischen Wasserstoffübertragung einer näheren Analyse unterwerfen.

Dabei dürfte vorausgeschickt werden, dass ein Vorgang, wobei unter dem Einflusse eines anorganischen Katalysators Wasserstoff aus einer organischen Substanz gelöst und auf eine zweite organische Substanz übertragen wird, auch in der reinen Chemie durchaus nicht unbekannt ist. Wir verweisen in diesem Zusammenhange nur auf die früher zitierte Abhandlung von Bredig und Sommer und auf eine Arbeit von Armstrong und Hilditch⁴³, deren Titel 'The Transference of Hydrogen from Saturated to Unsaturated Organic Compounds in the Liquid State in the Presence of Metallic Nickel' schon für sich spricht. Das Vermögen der lebenden Substanz zur Bewirkung einer derartigen Katalyse steht also gar nicht ohne Analogon in der leblosen Natur da.

Weitere Beispiele dafür sind übrigens in den von Wieland studierten Fällen der Oxydationskatalyse zu finden. So konnte dieser Forscher zeigen, dass die z.B. von den Essigbakterien bewirkte Übertragung von Wasserstoff aus Alkohol und Azetaldehyd (-hydrat) auf Sauerstoff oder Methylenblau auch mit Hilfe von Palladiumschwarz als Katalysator vor sich geht.

Wie können wir uns jetzt den Vorgang dieser Übertragungskatalyse näher vorstellen? Hierzu müssen wir zurückgreifen auf die Anschauungen der Chemiker über die rein chemische Katalyse. In dieser Hinsicht sind nun die von Böeseken⁴⁴ und Prins⁴⁵ entwickelten Anschauungen von der allergrössten Bedeutung.⁴⁶

In Übereinstimmung mit den von diesen beiden Forschern entwickelten Betrachtungen geht beim ersten Teil der Katalyse, d.h. bei

der Dehydrierung des Substrates durch das Protoplasma der katalysierenden Zelle ein Einfluss auf das Substrat aus, wobei diese Substanz eine Änderung erfährt, welche durch Böesen als Dislokation bezeichnet wird. Diese Änderung ist logischerweise eine Funktion der chemischen Affinitäten des Katalysators zu dem Dehydrierungssubstrat oder besser gesagt, zu in diesem Substrate vorhandenen Atomen. Indem ein Teil der Affinitäten dieser Atome von den Affinitäten des Katalysators beschlagnahmt wird, tritt in erster Phase eine Lockerung der Bindungen zwischen einzelnen Atomen im Molekül auf.

Es liegt nun auf der Hand, dass wir in dieser Beziehung an erster Stelle an die Affinität des Katalysators zu den Wasserstoff- und Sauerstoffatomen im Substrate denken müssen. Dabei muss von vornherein festgestellt werden, dass die Affinitäten einer Substanz zu Wasserstoff und Sauerstoff keine unabhängigen Eigenschaften sind, sondern derart zusammenhängen, dass zu einer grossen Affinität zu Sauerstoff immer eine kleine Affinität zu Wasserstoff gehört und umgekehrt, gerade wie dies sich auch im Verhalten der Metalle gegenüber Sauerstoff und Wasserstoff zeigt.

a. Überwiegende Affinität des Katalysators zu Wasserstoff

Dass eine Affinität des die Dehydrierung einleitenden Agens zu Wasserstoff zu einer Aktivierung von einem oder mehreren im Substrate anwesenden Wasserstoffatomen führen kann, möge auf folgende Weise erläutert werden. Die Sättigung eines Teiles der Affinität zu Wasserstoff von dem an und für sich ungesättigten Katalysator wird zur Folge haben, dass der Wasserstoff mit einer geringeren Kraft an den Substratrest gebunden bleibt. Hierdurch wird in diesem Rest ein Zustand von 'Ungesättigtheit' eintreten. Dieser Zustand wird inzwischen bewirken, dass die freigewordene Bindungskraft sich sonst irgendwo im Molekül zu sättigen versucht. Je nachdem diese Neigung befriedigt wird, oder je nachdem mehr oder weniger ausgesprochene Atomverschiebungen in dem Molekül stattfinden, wird der Wasserstoff mit einer geringeren Kraft an den Molekülrest gebunden bleiben, d.h. der Wasserstoff wird aktiviert. Im extremsten Fall wird dieser Zustand zu einem Übergang des Molekülrestes in eine in sich selbst gesättigte Verbindung führen, wobei dann die Bindung zwischen dem Wasserstoffatom und dem Molekülrest vollständig gelöst wird. In diesem Fall würden aber die Wasserstoffatome am Katalysator ge-

bunden bleiben, was jedoch bedeuten würde, dass die Reaktion keinen Fortgang nimmt. Dabei kann sich dann aber der Fall einstellen, dass die Bindungsneigung zweier Wasserstoffatome die Affinität des Katalysators überwindet, und die Reaktion unter Freiwerden gasförmigen Wasserstoffs fortschreitet.⁴⁷

Letzter Fall der Dehydrierung wird jedoch nur ausnahmsweise eintreten. In den meisten Fällen wird der Katalysator die Bindung des Wasserstoffs am Substratrest nur lockern. In diese Situation wird nur dann eine Änderung eintreten, wenn noch eine dritte Verbindung – der sogenannte Wasserstoffakzeptor – hinzukommt, welche ebenfalls freie Affinität zum Wasserstoff besitzt. In diesem Falle wird der Wasserstoff auf letztgenannte Verbindung übergehen können, wobei im Substratrest Affinitätsverschiebungen eintreten, welche zu einem neuen in sich gesättigten Zustande führen. Der Katalysator steht dann wieder für einen neuen Angriff zur Verfügung, worauf das Spiel der Bindungskräfte sich wiederholt.

Ob die geschilderte Wasserstoffübertragung wirklich stattfindet, wird nun von drei Faktoren bedingt werden: *a.* der Kraft, womit das zu aktivierende Wasserstoffatom im Substrat gebunden ist, *b.* der Bindungsneigung des Katalysators zu Wasserstoff, *c.* der Bindungsneigung des Akzeptors zu Wasserstoff. Wenn wir diesen letzten Faktor einen Augenblick konstant halten, indem wir einen Vergleich machen in Anwesenheit desselben Akzeptors bei der Dehydrierung zweier Substrate, worin die zu aktivierenden Wasserstoffatome mit verschiedener Kraft gebunden sind, dann leuchtet es sofort ein, dass das Substrat, worin der Wasserstoff am kräftigsten gebunden ist, auch einen Katalysator mit grösserer Affinität zu Wasserstoff fordert, um eine passende Aktivierung des Wasserstoffs zu bewirken, als das andere Substrat. Dabei ist jedoch zu bemerken, dass eine passende Wasserstoffaktivierung ebenso wenig erreicht wird, wenn die betreffende Affinität des Katalysators zu gross ist, denn dann wird das Wasserstoffatom eben zu kräftig an den Katalysator gebunden bleiben. In diesem Falle schiesst, wie Prins in diesem Zusammenhang bemerkt, der Katalysator, vom Standpunkt der Katalyse betrachtet, über das Ziel hinaus.

Die optimale Wasserstoffaktivierung wird nun in den verschiedenen Substraten, je nachdem das Wasserstoffatom, welches aktiviert wird, fester im Substrat gebunden ist, geringer sein, so dass dann für das

Gelingen der Katalyse auch ein kräftiger Wasserstoffakzeptor notwendig ist.

Das Prinzip obiger Anschauung, wonach die Aktivierung des Wasserstoffs eine Folge ist von der Bindungsneigung des Katalysators zu Wasserstoff, wird auch von Wieland⁴⁸ zur Erklärung seiner rein chemischen mit Hilfe von Platin oder Palladium bewirkten Katalysen herangezogen.

Inzwischen werden wir sehen, dass die Wasserstoffaktivierung ebenfalls eine Folge der freien Affinität des Katalysators zu Sauerstoff sein kann.

b. Überwiegende Affinität des Katalysators zu Sauerstoff

Dass eine überwiegende Affinität des Katalysators zu Sauerstoff ebenfalls zu einer Wasserstoffaktivierung führen kann, möge durch Folgendes erläutert werden. Betrachten wir zuerst die Dehydrierungssubstrate, worin das in einer Hydroxylgruppe vorhandene Wasserstoffatom aktiviert wird. In diesem Falle wird man auf Grund analoger Erwägungen, wie oben auseinandergesetzt, annehmen dürfen, dass eine Sättigung eines Teiles der Bindungsneigung des Sauerstoffatoms der Hydroxylgruppe von dem an sich ungesättigten Katalysator stattfindet, wodurch das Wasserstoffatom mit geringerer Kraft an das Sauerstoffatom gebunden bleibt, d.h. das Wasserstoffatom wird aktiviert. Die Anwesenheit eines genügend wasserstoffbegierigen Akzeptors wird dann in bestimmten Fällen genügen, um eine Übertragung des Wasserstoffs auf den Akzeptor stattfinden zu lassen. Das Losreißen des Wasserstoffs wird aber die Ursache sein, dass im Substratrest Affinitätsverschiebungen eintreten, wobei dieser in einen neuen gesättigten Zustand übergeht. Dabei wird die Bindung des Sauerstoffatoms mit dem Katalysator wieder gelöst, worauf dieser für einen neuen katalytischen Eingriff zur Verfügung steht.

In den Fällen, worin in sauerstoffhaltigen Substraten kein Wasserstoff direkt an den Sauerstoff gebunden ist, wird unter Umständen ebenfalls eine Wasserstoffaktivierung eintreten können. Um dies einzusehen, braucht man nur zu realisieren, dass die Beschlagnahme eines Teils der Bindungsneigung des Sauerstoffs durch den Katalysator zur Folge haben wird, dass die Bindung des Sauerstoffatoms am Molekülrest geschwächt wird. Das Atom des Restes, woran das Sauerstoffatom gebunden ist, wird deshalb ebenfalls ungesättigt werden und

daher versuchen, anderswo im Molekül Affinitäten zu beschlagnehmen. Die Folge dieses Vorganges wird eine Lockerung der Bindungen von Wasserstoffatomen, d.h. eine Wasserstoffaktivierung sein können.⁴⁹

Wir legen grossen Wert darauf, hier festzustellen, dass eine ähnliche Erklärung der Wasserstoffaktivierung durch eine Affinität des Katalysators zu Sauerstoff bereits von Prins für die von ihm studierten Reduktionsprozesse gegeben wird.⁵⁰

Aus obigen Auseinandersetzungen folgt nun, dass wenigstens bei den sauerstoffhaltigen Dehydrierungssubstraten die Wasserstoffaktivierung sowohl bewirkt werden kann durch die Bindungsneigung des Katalysators zu Wasserstoff als durch diejenige zu Sauerstoff. Da jedoch eine grosse Affinität des Katalysators zu Wasserstoff immer eine kleine Affinität zu Sauerstoff bedeutet und umgekehrt, wird in der Regel nur einer dieser beiden Faktoren praktische Bedeutung haben.⁵¹

Zu diesem allen müssen wir nur noch hinzufügen, dass in bestimmten Fällen der Katalysator ebenfalls Affinitäten von Wasserstoff- oder Sauerstoffatomen in dem als Akzeptor auftretenden Molekül beschlagnehmen wird, was sich unter Umständen in einer Verstärkung der akzeptierenden Wirkung äussern wird. Dabei ist zu beachten, dass dadurch bisweilen einander entgegenarbeitende Tendenzen entstehen, indem zwar eine grössere Wasserstoffaffinität des Protoplasmas auch bei optimaler Aktivierung des Wasserstoffs einen kräftigeren Akzeptor fordert, aber gleichzeitig diese Affinität auch eine kräftigere Aktivierung eines an sich wenig kräftigen Akzeptors bedingen kann.

4. ANWENDUNG DER GEGEBENEN VORSTELLUNG DES CHEMISMUS DER KATALYTISCHEN WASSERSTOFFÜBERTRAGUNG AUF DIE ZUCKERDISSIMILATIONSPROZESSE

Wir wollen jetzt näher betrachten, inwieweit die im vorigen Teil gegebene Hypothese über den Chemismus der katalytischen Wasserstoffübertragung sich eignet, um angewendet werden zu können bei der Erklärung der im 2. Teil behandelten Dissimilationsprozesse, wobei Zucker als Substrate fungieren.

Wir sahen, dass bei unseren Ausführungen die für die katalytische Wasserstoffübertragung unentbehrliche Wasserstoffaktivierung sowohl die Folge einer Affinität des Katalysators zu Wasserstoff als die

einer Affinität zu Sauerstoff sein kann. Wir wollen uns jetzt die Frage vorlegen, ob es Gründe gibt anzunehmen, dass diese Vorstellung auch bei den oben genannten biochemischen Katalysen zutrifft.

Hierbei ist das die Katalyse bewirkende Agens das Protoplasma⁵² der betreffenden Zelle. Wenn wir uns jetzt fragen, ob irgendwelche Anzeichen vorhanden sind, dass das Protoplasma der verschiedenen Zellen freie Affinität zu Sauerstoff oder zu Wasserstoff hat, dann stossen wir sofort auf die ausserordentliche Sensibilität der obligat anaeroben Organismen dem freien Sauerstoff gegenüber. Wenn man bedenkt, dass äusserst geringe Quantitäten Sauerstoff den Tod dieser Organismen in sehr kurzer Zeit herbeiführen, dann ist man selbstverständlich gezwungen, hierbei an eine chemische Wirkung dieser Substanz zu denken. Es liegt dann auf der Hand, an eine Bindung des Sauerstoffs durch das die Dissimilation bewirkende Agens, d.h. durch das Protoplasma, zu denken, wodurch der für das Leben unentbehrliche Dissimilationsprozess ausgeschaltet wird. Hier ist also die Annahme einer Affinität des Protoplasmas zu Sauerstoff durchaus motiviert, und die Wasserstoffübertragung bei den obligat anaeroben Organismen lässt sich ungezwungen dieser Affinität zuschreiben. Unter den von uns besprochenen Gruppen gilt dies für die Gruppe der Buttersäure- und Butylalkoholbakterien, deren Dissimilation also eine Folge der ausgesprochenen Affinität des Protoplasmas dieser Bakterien zu Sauerstoff ist. Aber diese Sachlage trifft auch für die Gruppen der Propionsäurebakterien und der wahren Milchsäurebakterien zu, deren Vertreter zwar in gewöhnlichem Sinne nicht obligat anaerob sind, sich jedoch nicht nur am üppigsten bei Sauerstoffausschluss vermehren, sondern in vielen Fällen sogar ausgesprochen luftscheu sind. Dass diese Bakterien anscheinend dem freien Sauerstoff gegenüber resistent sind, findet seine Erklärung einmal darin, dass die Affinität des Protoplasmas zu Sauerstoff hier geringer ist als bei den obligat anaeroben Organismen, dann in dem Umstand, dass die Sauerstoffempfindlichkeit durchweg beurteilt wird nach Versuchen bei gleichzeitiger Anwesenheit guter Dissimilationssubstrate. In diesem Falle wird nicht das freie Protoplasma dem Angriffe des Sauerstoffs ausgesetzt sein, sondern das Protoplasma wird jetzt geschützt von den aktivierten Wasserstoffatomen des an ihn locker gebundenen Substrates. Diese Atome werden dann vom Sauerstoff ak-

zeptiert, wodurch die Gefahr abgelenkt wird und der Dissimilationsvorgang einfach teilweise in andere Bahnen geführt wird.

Diese Vorstellung gibt gleichzeitig eine Erklärung für die von Berthelot⁵³ festgestellte, aber nicht aufgeklärte Tatsache, dass eine Zugabe von brenztraubensauren Salzen die Kultur von sogar obligat anaeroben Organismen in offenen an der Luft stehenden Gefäßen ermöglicht. Eben weil diese Salze oder der daraus allmählich gebildete Azetaldehyd leicht zu aktivierenden Wasserstoff enthält, wird der von den Anaerobiern kräftig aktivierte Wasserstoff das Protoplasma vor dem Sauerstoff der Luft schützen.

Für alle weiteren von uns betrachteten Gruppen liegt nun kein Grund vor, an eine direkte Bindung des Sauerstoffs an das Protoplasma zu denken. Im Gegenteil weist der Umstand, dass bei allen diesen Organismen das Protoplasma zu einer katalytischen Zerlegung von Wasserstoffsuperoxyd unter Sauerstoffentwicklung imstande ist, darauf hin, dass das wasserstoffübertragende Agens keine ausgesprochene freie Affinität zu Sauerstoff besitzt.

Es liegt nun auf der Hand anzunehmen, dass bei diesen Gruppen von aeroben oder fakultativ aeroben Organismen die freie Affinität des Protoplasmas zu Wasserstoff der Faktor ist, welche hier die katalytische Wasserstoffübertragung bewirkt.

Hierfür spricht an erster Stelle die Tatsache, dass die Essigbakterien die katalytische Dehydrierung von Alkohol zu Azetaldehyd und von dieser Substanz zu Essigsäure bewirken, was auch mit Hilfe von Palladiumschwarz vorgenommen werden kann. Und für die Erklärung dieser letzten Katalyse zieht Wieland, wie schon bemerkt, ebenfalls die freie Affinität des Palladiums zu Wasserstoff heran.

Deutlicher noch spricht die Berechtigung der Annahme einer freien Affinität des Protoplasmas zu Wasserstoff bei den aeroben Organismen aus der Tatsache, dass man in der letzten Zeit festgestellt hat, dass es zahlreiche Bakterien gibt, welche imstande sind, gasförmigen Wasserstoff zu verbrennen.⁵⁴

Das wichtigste Ergebnis der neueren Arbeiten ist zweifellos, dass diese biokatalytische Umsetzung des Knallgases nicht, wie man bis vor kurzem annahm, eine merkwürdige Eigenschaft weniger ganz bestimmter Bakterienarten ist,⁵⁵ sondern dass Bakterien aus ganz verschiedenen Gruppen und zwar solchen, die auch zu ganz anderen Dissimilationsprozessen imstande sind, unter Umständen auch den gas-

förmigen Wasserstoff verarbeiten. In diesem Zusammenhang muss bemerkt werden, dass Visser 't Hooft neulich in diesem Laboratorium nachweisen konnte, dass es auch typische Essigbakterien gibt, die zu dieser Katalyse befähigt sind.⁵⁶

Es leuchtet sofort ein, welche wichtige Stütze die Erklärung der Dissimilationsprozesse auf Grund des Wasserstoffaktivierungsprinzips hierdurch findet. Denn es geht hieraus hervor, dass die Wasserstoffoxydation durchaus den andern von diesen Organismen bewirkten Oxydationen gleichzustellen ist. Überlegt man dazu, dass für die Knallgaskatalyse mit Hilfe von Palladium sehr viel zugunsten der Auffassung spricht, dass dieser Katalysator die Spaltung des molekularen Wasserstoffs durch seine freie Affinität zu elementarem Wasserstoff bewirkt, dann scheint es durchaus berechtigt, dieses Erklärungsprinzip auch bei den sonstigen Dissimilationsprozessen heranzuziehen. Dass eben nicht alle mit Wasserstoffaffinität ausgerüsteten Zellen zu einer Aktivierung und einer darauf anschliessenden Übertragung der Atome gasförmigen Wasserstoffs befähigt sind, ist eine logische Konsequenz der gegebenen Theorie der Katalyse. Denn auch im Wasserstoffmolekül sind die Atome mit einer bestimmten Kraft gebunden und diese Atome werden nur von einem Katalysator mit einer ganz bestimmten Bindungsneigung für Wasserstoff genügend aktiviert. Sowohl ein Über- wie ein Untermass wird wieder schädlich sein für die Katalyse, die unter Umständen dadurch ganz fortbleiben kann.

Die obigen Ausführungen werden genügen, um die Annahme zu berechtigen, dass die Dissimilationsprozesse in den ersten sechs der im 2. Teil behandelten Gruppen eine direkte Folge sind von der Affinität des Protoplasmas der Zellen zu Wasserstoff. Und zwar liegt es auf der Hand vorauszusetzen, dass diese Affinität bei der ersten Gruppe am grössten ist, um von diesen ausgesprochen aeroben Organismen ausgehend immer niedriger zu werden, bis schliesslich bei den Milchsäurebakterien ein Überwiegen der Sauerstoffaffinität eintritt.

Es bedarf wohl kaum näherer Auseinandersetzung, dass wir in den Unterschieden der Grösse der Wasserstoffaffinität bei den verschiedenen Gruppen die Ursache sehen, dass die Zuckerdissimilation bei diesen Gruppen so verschiedene Wege einschlägt. Wenn man diese Unterschiede voraussetzt, ist es eine logische Konsequenz der im 3. Teil entwickelten Auffassung über den Chemismus der katalytischen Wasserstoffübertragung, dass eine bestimmte Wasserstoffaffinität des

Protoplasmas geeignet ist, eine optimale Wasserstoffaktivierung zu bewirken bei Wasserstoffatomen, die mit einer bestimmten Kraft im Substrat gebunden sind. Eine grössere oder eine kleinere Wasserstoffaffinität des Protoplasmas wird zur Folge haben, dass die in Betracht kommenden Wasserstoffatome entweder gar nicht oder in ungenügender Weise aktiviert werden, so dass damit die Katalyse nicht oder jedenfalls mit viel geringerer Geschwindigkeit stattfindet. Bei den betrachteten wasserstoffreichen Substraten wird aber eine solche grössere oder kleinere Wasserstoffaffinität in vielen Fällen eben eine optimale Aktivierung bewirken im Gegensatz zu den zuerst betrachteten Wasserstoffatomen, d.h. die katalytische Wasserstoffübertragung wird einen anderen Weg einschlagen.

In unserer Auffassung ist also die grosse Verschiedenheit, die der Zuckerabbau in den verschiedenen Gruppen aufweist, in erster Linie auf eine quantitative Änderung einer einzigen Eigenschaft des Protoplasmas zurückzuführen.

Ganz in Übereinstimmung mit unserer Ansicht, dass bei den aeroben Gruppen, bei denen die Wasserstoffaffinität am grössten ist und infolgedessen die *optimale* Wasserstoffaktivierung stets kleiner als die *optimale* Wasserstoffaktivierung der Gruppen mit geringerer Wasserstoffaffinität, steht die Tatsache, dass bei den ersten Gruppen stets sehr kräftige Wasserstoffakzeptoren – normalerweise der nach Warburg aktivierte Luftsauerstoff oder auch Methylenblau – zum Gelingen der Katalyse notwendig sind. Für die Organismen mit geringerer Wasserstoffaffinität genügen immer schwächere Akzeptoren für die Wasserstoffübertragung. Hierdurch wird auch erklärlich, dass bei den mehr aeroben Organismen niemals gasförmiger Wasserstoff in den Stoffwechsel auftritt.

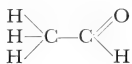
Gegen die oben entwickelte Auffassung, dass jede bestimmte Wasserstoffaffinität vor allem ganz bestimmte Katalysen bewirkt, könnte man nun anführen, dass es bestimmte Teilreaktionen gibt, welche bei vielen Gruppen auftreten, wie z. B. die Milchsäurebildung bei Gruppe II bis IX. Dazu muss bemerkt werden, dass es sich bei diesen Reaktionen vor allem um *intramolekulare* Umlagerungen und Kondensationsreaktionen handelt. Bei einer näheren Betrachtung kann es nun gar nicht wundern, dass in diesen Fällen die Katalyse in hohem Grade unabhängig ist von der Wasserstoffaffinität des Protoplasmas; denn hierbei wird eine kräftigere Bindung des Wasserstoffatoms vom

Protoplasma notwendigerweise zur Folge haben, dass auch der betreffende Akzeptor – bei der intramolekularen Umlagerung der Molekülrest – bei den Kondensationen ein zweites Substratmolekül, mehr ungesättigt wird und also kräftigere Akzeptorwirkung entfalten wird.

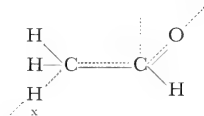
Es ist nicht möglich, hier für alle in unserem Schema aufgenommenen Katalysen eine nähere Betrachtung anzustellen, wie eine spezielle Katalyse unter dem Einflusse der Wasserstoff- bzw. der Sauerstoffaffinität des Protoplasmas vor sich geht.

Als Beispiel wollen wir nur die verschiedenartigen Umwandlungen des Azetaldehyds, der in allen Gruppen als Zwischenprodukt beim Zuckerabbau⁵⁷ auftritt, einer näheren Betrachtung unterwerfen.

Bekanntlich hat der Azetaldehyd folgende Strukturformel:

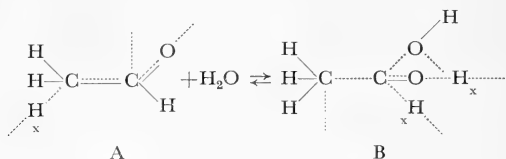


Inzwischen gibt diese Formel ungenügende Auskunft über die präzise Affinitätsverteilung im Molekül. Die grosse Neigung des Azetaldehyds zu Anlagerungen an die CO-Gruppe, lässt erkennen, dass die unten folgende Vorstellung zweifellos die Natur des Azetaldehyds besser wiedergibt. Dabei sind schwächere Bindungen als eine normale Valenz mit \cdots , stärkere Bindungen mit \equiv wiedergegeben. In welchem Masse die Schwächung oder die Verstärkung der verschiedenen Bindungen auftritt, lässt sich nicht sagen. Nur ist anzunehmen, dass die drei H-Atome in der CH_3 -Gruppe nicht mit gleicher Kraft gebunden sind, und dass eine Beschlagnahme der Affinität dieser Kohlenstoffatome sich vor allem in einer Lockerung des am schwächsten gebundenen H-Atomes äussern wird. Daher können wir uns den Azetaldehyd wie folgt denken:



Das ungesättigte Sauerstoffatom wird nun bewirken, dass das Azetaldehyd in verdünnter wässriger Lösung zum Teil ein Molekül Wasser addiert. Die Anlagerung der OH-Gruppe an das Kohlenstoffatom und des Wasserstoffatoms an das Sauerstoffatom wird jedoch nur locker sein. Dieses letzte Atom wird immer noch mehr als eine Valenz

des Kohlenstoffatoms in Anspruch nehmen, wodurch die sonstigen Bindungen dieses Kohlenstoffatoms gelockert werden. Folglich können wir das auftretende Gleichgewicht wiedergeben als:



Bei allen weiteren Betrachtungen muss also mit dem Vorhandensein dieser beiden Reaktionsformen des Azetaldehyds gerechnet werden.

Eine kräftige Wasserstoffaffinität, wie die der Essigbakterien z.B., hat offenbar zur Folge, dass die beiden prädisponierten (mit x ange deuteten) H-Atome der Form B in passender Weise aktiviert werden, um eine Übertragung auf einen kräftigen Wasserstoffakzeptor zu ermöglichen, bei Anwesenheit des (nach Warburg aktivierten) Sauerstoffs auf diesen. Dabei wird dann neben Wasser Essigsäure gebildet. Fehlt aber dieser Wasserstoffakzeptor, dann wird auch die A-Form des Azetaldehyds als Akzeptor genügen, wie Neuberg und Windisch⁵⁸ neulich gezeigt haben, und die Folge wird eine Cannizzaro-Umlagerung oder Dismutation des Azetaldehyds sein, wobei gleiche Teile Äthylalkohol und Essigsäure gebildet werden.

Bei wahrscheinlich noch kräftigerer Wasserstoffaffinität des Protoplasmas wird ausser den aktiven H-Atomen der Form B auch das prädisponierte H-Atom der Form A genügend aktiviert werden, um zur Katalyse geeignet zu sein. Diese Aktivierung bedeutet aber zu gleicher Zeit eine kräftige Verstärkung der 'Akzeptorwirkung' des O-Atomes in Form A. Das Resultat ist eine katalytische Übertragung des aktivierten Wasserstoffatoms eines Moleküls A auf das Sauerstoffatom eines zweiten Moleküls A. Die dadurch ungesättigt gewordenen C-Atome lagern sich aneinander, und eine Aldolkondensation findet statt.⁵⁹

Bei den Gruppen mit weniger kräftiger Wasserstoffaffinität tritt neben der Dehydrierung von Form B und Übertragung der betreffenden Wasserstoffatome auf Sauerstoff oder Azetaldehyd (Dismutation) noch eine andere Katalyse ein. Indem die Wasserstoffaffinität offenbar nicht ausreicht, um *beide* prädisponierten Wasserstoffatome

der Form B optimal zu aktivieren, beschränkt die Aktivierung sich hier fast nur auf das an das C-Atom gebundene H-Atom. In diesem Fall wird nur dieses eine H-Atom auf das O-Atom der Form A übertragen und die beiden dadurch ungesättigt gewordenen C-Atome (respektive der Form A und der Form B) lagern sich aneinander: es findet eine Kondensation des Azetaldehyds zu Azetylmethylkarbinol (Azetoin) statt. Hierdurch wird begreiflich, dass zwei zweifellos so nah verwandte Bakterienarten, wie *B. coli* und *B. aerogenes* augenscheinlich so verschiedene Zucker-Dissimilationsprozesse aufweisen. Jetzt ist es aber klar, dass die für das letztere Bakterium charakteristische Azetylmethylkarbinol-kondensation eben nur eine unvollständig gelungene, für *B. coli* typische Dismutation des Azetaldehyds bedeutet.

Ferner hat man bei all diesem zu beachten, dass zwar eine bestimmte Wasserstoffaffinität des Protoplasmas eine ganz bestimmte Wasserstoffübertragung vorzugsweise katalysiert, dass man aber daraus nicht schliessen darf, dass das Protoplasma nicht auch andere Wasserstoffübertragungen katalysieren kann, wenn die bevorzugte Reaktion aus irgend einem Grunde nicht eintreten kann.

Während z. B. bei der alkoholischen Gärung der Azetaldehyd (in der A-Form) normalerweise ganz als Akzeptor für den im Methylglyoxalhydrat aktivierten Wasserstoff in Anspruch genommen wird, tritt bei kräftiger Aeration der (aktivierte) Luftsauerstoff als Akzeptor für den genannten Wasserstoff auf, so dass der Azetaldehyd von dem Protoplasma der Hefe in andere Richtungen verarbeitet werden kann.⁶⁰ Einerseits wird der Azetaldehyd zu Kohlensäure und Wasser dehydriert, andererseits wird er zu Azetylmethylkarbinol kondensiert. Ausserdem wird jedoch unter den genannten Bedingungen der Luftsauerstoff aller Wahrscheinlichkeit nach schon vorangehend an der Azetaldehydbildung in das Spiel der Reaktionen eingreifen. In diesen Änderungen des normalen Gärungsvorganges unter dem Einflusse des Sauerstoffs liegt eben das Geheimnis der Lufthefefabrikation. Auch durch Zufügung anderer geeigneter Wasserstoffakzeptoren zu einer gärenden Zuckerlösung kann man dafür sorgen, dass die Akzeptorfunktion des Azetaldehyds teilweise ausgeschaltet wird.⁶¹

Eine geringe Sauerstoffaffinität des Protoplasmas wird nun ebenfalls eine Aktivierung der prädisponierten H-Atome in den beiden Formen des Azetaldehyds zur Folge haben, so dass auch hier noch Dehydrierung der Form B und Übertragung auf gleichzeitig vorhandene

Wasserstoffakzeptoren unter Essigsäurebildung vorkommt. Bei einer grösseren Sauerstoffaffinität wird offenbar ein Freikommen von den Wasserstoffatomen aus Form B unter Bildung gasförmigen Wasserstoffs sehr gefördert, wie dies von uns für die Buttersäurebakterien durchaus wahrscheinlich gemacht worden ist.⁶² Bei Form A wird die Sauerstoffaffinität ebenfalls eine kräftige Aktivierung des prädisponierten Wasserstoffatoms bewirken, da aber hier der eventuell entstehende Molekülrest nicht existenzfähig ist, kann eine Übertragung dieses Wasserstoffatoms nur auf ein zweites Molekül unter gleichzeitiger Anlagerung der betreffenden Kohlenstoffatome, also unter Aldolkondensation stattfinden. Diese Reaktion ist dann der Ausgangspunkt für die bei den betreffenden Organismen so vorherrschende Buttersäure- (und Butylalkohol-) bildung.

5. ANWENDUNG DER WASSERSTOFFÜBERTRAGUNGSTHEORIE AUF SONSTIGE DISSIMILATIONSPROZESSE

Im vorigen Teil haben wir die verschiedensten Zucker-Dissimilationsprozesse zu erklären versucht durch eine einzige Eigenschaft des Protoplasmas, die von Fall zu Fall quantitativ verschieden ist. Es drängt sich nun die Frage auf, ob eine ähnliche Vorstellung auch für die speziell in der Mikrowelt deutlich ans Licht tretenden Dissimilationsprozesse anderer Substrate zutrifft.

Wir sind der Ansicht, dass dies ebenfalls möglich ist. Betrachten wir hierzu zuerst die weiteren oxydativen Dissimilationsprozesse. Es leuchtet sofort ein, dass wir bei allen diesen Prozessen die vereinigte Wieland-Warburgsche Anschauung (Oppenheimers 'Einheitliche Deutung') ungehindert anwenden können. Die Frage ist nur, ob es auch hierbei genügt, nur eine einzelne Eigenschaft des Protoplasmas zur Erklärung des Verhaltens der verschiedenen Organismen zu den verschiedenen Dissimilationssubstraten heranzuziehen.

Beim ersten Anblick scheint die ausgesprochene Spezifität der verschiedenen Organismen sich einer solchen Auffassung zu widersetzen. Zwar könnte man z. B. das ausgesprochene Vermögen verschiedener Mykobakterien⁶³ zur Dehydrierung der von den meisten Organismen nicht angegriffenen Kohlenwasserstoffe (wie Benzin, Erdöl u. dgl.) dadurch erklären, dass in diesen Substraten mit kräftig gebundenen Wasserstoffatomen nur Wasserstoffaktivierung auftritt,

wenn das Protoplasma, wie dies dann bei den betreffenden Mykobakterien der Fall sein würde, eine sehr grosse Wasserstoffaffinität besitzt. Doch begegnet man bei einer solchen Auffassung in anderen Fällen Schwierigkeiten.

Es ist z. B. bekannt, dass verschiedene Bakterien der *Pseudomonas*-Gruppe vor allem angepasst sind an eine oxydative Verarbeitung von Produkten der Eiweisshydrolyse. Doch sind dieselben auch imstande, anstatt dieser Produkte Salze von organischen Säuren als Dissimilationssubstrat zu benutzen. Dieser letzten Eigenschaft begegnet man aber ebenfalls in ausgesprochenem Masse bei den verschiedensten Essigsäurebakterien,⁶⁴ weshalb man auf eine gleiche oder nahezu gleiche Wasserstoffaffinität bei diesen beiden Bakteriengruppen schliessen muss. Damit scheint jedoch die Tatsache, dass die *Pseudomonas*-Arten mit Zucker und Alkoholen, die *Acetobacter*-Arten mit Peptonen als Dissimilationssubstrat schlecht oder gar nicht auskommen, unvereinbar.

Wir sind jedoch der Ansicht, dass dieser Widerspruch nur scheinbar ist. Unsere im 3. Teil gegebene Anschauungsweise des Chemismus der katalytischen Wasserstoffübertragung ist nämlich in einer Hinsicht absichtlich unvollständig gewesen. Wir haben uns nämlich darauf beschränkt, eine Vorstellung zu geben, nach welcher die Wasserstoffaffinität im Substrat, die Wasserstoffaffinität des Katalysators und die Bindungsneigung des Akzeptors zu Wasserstoff – die drei Faktoren, welche über das Zustandekommen der Katalyse entscheiden – unveränderliche Grössen sind. In Wirklichkeit wird dies aber nicht zutreffen; denn die physische Chemie lehrt, dass diese Eigenschaften, die in den Oxydoreduktionspotentialen der genannten Substanzen ihren quantitativen Ausdruck finden, in hohem Grade abhängig sind von der herrschenden Wasserstoffionenkonzentration.⁶⁵

Nun brauchen wir nur noch daran zu denken, dass einerseits jede oxydative Verarbeitung von hydrolysierten Eiweisspaltprodukten eine Produktion von Ammoniak zur Folge hat, und die Dissimilation sich also in einem alkalischen Medium abspielt, dass andererseits die oxydative Verarbeitung von Zucker und Alkoholen immer mit intermediärer Produktion von Säuren zusammengeht, um einzusehen, dass die nahezu beim Neutralpunkte vor sich gehende Verarbeitung der organischen Salze durch beide Bakteriengruppen gar nicht einzuschliessen braucht, dass beide sich auch gegenüber den weitergenannten Substraten ähnlich verhalten werden.

Wenn wir dies alles berücksichtigen, so steht vorläufig der Annahme nichts im Wege, dass der ganze Unterschied im Verhalten der einzelnen Organismen den verschiedenen Dissimilationssubstraten gegenüber seine Ursache darin findet, dass das Protoplasma eine Wasserstoff- oder Sauerstoffaffinität besitzt, die für eine jede Art in einem charakteristischen Zusammenhange mit der Wasserstoffionenkonzentration steht und sowohl bei zu niedriger wie bei zu hoher Konzentration dieser Ionen auf Null herabsinkt. Dabei sind die Grenzen des zulässigen Wasserstoffionengebietes für jede Art verschieden. Diese Auffassung findet, wie man einsehen wird, eine Parallele in dem Zusammenhang zwischen Enzymwirkung und Wasserstoffionenkonzentration, der in den klassischen Arbeiten von Sörensen, Michaelis u.a. festgestellt worden ist.

In Übereinstimmung mit dieser Ansicht steht nun die Tatsache, dass eine Änderung der Wasserstoffionenkonzentration innerhalb des für einen Organismus zulässigen Gebietes zur Folge hat, dass bestimmte Wasserstoffübertragungskatalysen ausfallen und andere derartige Katalysen dafür an deren Stelle treten. Das schönste Beispiel dafür ist die bekannte von Neuberg entdeckte sogenannte dritte Vergärungsform des Zuckers bei der alkoholischen Gärung. Während die Alkoholhefe normalerweise den Wasserstoff des Methylglyoxalhydrats auf Azetaldehyd überträgt, tritt in einem alkalischen Medium Glyzerinaldehyd als Akzeptor in den Vordergrund, wobei dann der Azetaldehyd ebenfalls teilweise zu Essigsäure dehydriert wird und der daraus entstammende Wasserstoff auf ein zweites Molekül Aldehyd übertragen wird, unter Bildung von Äthylalkohol.

Wir haben weiter feststellen können, dass es gelingt, die Zuckervergärung durch *B. coli* durch Pufferung des Mediums derartig zu verändern, dass der Azetaldehyd nicht, wie es normalerweise stets der Fall ist, in Äthylalkohol und Essigsäure, sondern teilweise in Azetylmethylkarbinol (also wie bei *B. aerogenes*) übergeführt wird.

Als drittes Beispiel möge angeführt werden, dass Kostytschew und Afanassjewu gezeigt haben, dass Schimmelpilze, wie *Penicillium glaucum*, die unter anaeroben Bedingungen normalerweise aus Zucker keine Spur Äthylalkohol bilden, hierzu sehr merkbar befähigt werden, wenn man den Versuch in schwach alkalischer Lösung vornimmt.⁶⁶

Diese Beispiele sind noch durch viele andere zu vermehren, doch wird das obige genügen, um unsere Anschauungsweise zu rechtfertigen.

Wir wollen nun noch mit einigen Beispielen zeigen, wie fruchtbar die allgemeine, katalytische Wasserstoffübertragungstheorie in ihrer Anwendung auf die verschiedensten Dissimilationsprozesse ist.

I. Der Nitrifikationsvorgang

Bekanntlich steht es seit den klassischen Untersuchungen Winogradskys – die in denjenigen Meyerhofs⁶⁷ einen wichtigen Abschluss gefunden haben – fest, dass der Nitrifikationsvorgang, d. h. der Übergang der Ammonsalze in Nitrate, in zwei Stufen verläuft. Dabei wird jede dieser beiden Stufen, d. h. die Oxydation der Ammonsalze zu Nitriten und die Oxydation der Nitriten zu Nitraten von einer spezifischen Bakterienart bewirkt.

Aus den genannten Untersuchungen geht klar hervor, dass die beiden Bakterienarten aus den von ihnen bewirkten anorganischen Oxydationsvorgängen ihre Energie nehmen und dass dieselben die Dissimilationsprozesse dieser Organismen darstellen.

Meistens findet man diese Vorgänge wie folgt wiedergegeben. Für das Nitritbakterium:

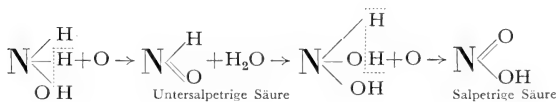
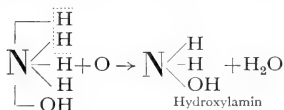


und für das Nitratbakterium:

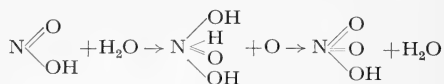


Können wir nun die katalytische Wasserstoffübertragungstheorie auch auf diese Prozesse anwenden? Wie wir sehen werden, ist dies durchaus möglich und wir erreichen damit den Vorteil, dass die enge Verwandtschaft dieser Vorgänge mit den normalen Atmungsprozessen deutlich hervortritt.

Es ist nun wahrscheinlich, dass der Dissimilationsprozess der Nitritbakterien sich folgenderweise abspielen wird:



Der Dissimilationsprozess der Nitratbakterien könnte dann wie folgt wiedergegeben werden:



Diese Vorstellungsweise bringt nun mit, dass für die Nitritbakterien Hydroxylamin und untersalpetrige Säure (oder dessen Anhydrid N_2O) als Zwischenprodukte bei der Nitritbildung auftreten. Die Prüfung der Richtigkeit dieser Hypothese wird in diesem Laboratorium vorgenommen. Die Frage ist deshalb nicht ohne Bedeutung, weil Winogradsky trotz mannigfacher Versuche die oxydierende Wirkung der Nitritbakterien als streng spezifisch für Ammonsalze gefunden hat. Die oben genannten Zwischenprodukte sind jedoch, soweit bekannt, bisher nicht untersucht worden.

Weiter eignet sich die Wasserstoffübertragungstheorie noch dazu, eine ganz andere Eigenschaft dieser Bakterien begreiflich zu machen. Bekanntlich verläuft die rein chemische Oxydation der Ammonsalze viel schwieriger als die Oxydation der sonst in Frage kommenden organischen Dissimilationssubstrate. Daher wird die Wasserstoffaktivierung in den Ammonsalzen nach unserer Auffassung eine überaus grosse Wasserstoffaffinität des Protoplasmas der Nitritbakterien fordern. Es ist nun verführerisch, in diesem Umstände die Ursache zu erblicken, dass die Nitrifikationsbakterien bekanntlich nicht imstande sind die üblichen organischen Dissimilationssubstrate zu benutzen und dass diese Substanzen die Nitrifikation verzögern und zwar um so mehr, je besser diese Substanzen für andere lebenden Zellen angreifbar sind (Winogradsky).⁶⁸ Eben weil diese gut angreifbaren Substanzen leichter aktivierbare Wasserstoffatome enthalten, werden diese vom Protoplasma der Nitritbakterien zu fest gebunden um übertragen werden zu können, und damit wird auch die Übertragung der Wasserstoffatome aus dem Ammoniak verhindert.

In der Abneigung dieser extrem oxydativen Organismen leicht angreifbaren organischen Substanzen gegenüber, könnte man also gewissermassen ein Gegenstück sehen zu der Sensibilität der obligat anaeroben Organismen gegenüber freiem Sauerstoff oder Verbindungen, welche locker gebundenen Sauerstoff enthalten.

II. Die Schwefelbakterien

Wir werden bei der Verwendungsmöglichkeit der katalytischen Wasserstoffübertragungstheorie für die Erklärung der Dissimilationsprozesse dieser Gruppe nicht lange verweilen. Wenn wir uns auf die Oxydation des Schwefelwasserstoffs zu freiem Schwefel beschränken, der von den verschiedensten Vertretern dieser physiologischen Gruppe entweder interzellulär oder extrazellulär abgeschieden wird, tritt die katalytische Wasserstoffübertragung vom Schwefelwasserstoff auf den Sauerstoff sofort deutlich ans Licht. Aber auch für die weitere Oxydation des Schwefels zu Schwefelsäure ist eine Dehydrierung nach vorhergehender Hydratisierung durchaus annehmbar.

Die Wasserstoffübertragungstheorie bietet aber grosse Vorteile bei der Betrachtung des Dissimilationsprozesses der äusserst interessanten schwefelhaltigen Purpurbakterien, die merkwürdigerweise zweifellos bei Abwesenheit von freiem Sauerstoff den Schwefelwasserstoff und den Schwefel zu Sulfat oxydieren. Dazu sind sie aber nur mit Hilfe der Lichtenergie imstande. Sowohl von Winogradsky, wie von Engelmann, Molisch u.a. sind nun vergebens Versuche gemacht worden um nachzuweisen, dass die Abhängigkeit dieser Bakterien vom Lichte darauf zurückzuführen sei, dass das Licht eine Zerlegung der Kohlensäure unter Bildung des freien Sauerstoffs bewirkt. Niemals ist es aber gelungen, die Produktion von Sauerstoff auch unter den verschiedensten Bedingungen einwandfrei nachzuweisen. Auch in den neuesten Arbeiten auf diesem Gebiete von Bavendamm⁶⁹ und von Baas Becking⁷⁰ wird an dieser Auffassung festgehalten. Dies ist, was diesen letzten Forscher betrifft, um so bemerkenswerter, als er die Wielandsche Ansicht zur Erklärung der Kohlensäureassimilation bei diesen Bakterien heranzieht. Wenn man diese Betrachtungsweise einmal akzeptiert, fällt aber die Notwendigkeit, dass der Akzeptor bei der Dehydrierung des Schwefelwasserstoffes oder des Schwefels gerade Sauerstoff sein soll, weg; denn es kann ebensogut irgend ein anderer Akzeptor im Spiele sein. Nur muss das Prinzip beibehalten bleiben, dass die gebildete Akzeptor-Wasserstoffverbindung mit Hilfe der Lichtenergie imstande ist, den Wasserstoff auf die Kohlensäure zu übertragen.⁷¹

Nebenbei möge bemerkt werden, dass wir uns nicht der Ansicht Baas Beckings anschliessen können, dass aus der Tatsache, dass die freie Energie des Schwefels höher ist als die des im Wasser gelösten

Schwefelwasserstoffs, folgen würde, dass nicht die Oxydation dieser letzten Substanz, sondern die des SH-Ions die energieliefernde Reaktion sein würde. Das zweite Hauptgesetz der Thermodynamik fordert nur, dass die freie Energie des Systems $\text{H}_2\text{S aq} + \text{Akzeptor}$ höher ist als die freie Energie des Systems $\text{S} + \text{Akzeptorwasserstoff}$. Dabei kann aber die freie Energie der einen Komponente (S) des zweiten Systems sehr wohl höher sein als die freie Energie einer der beiden Komponenten des ersten Systems (H_2Saq). Wie wir im nächsten Teil sehen werden, ist dieser Fall auch bei den verschiedensten Dissimilationsreaktionen nicht selten.

Dies hindert nicht, dass wir es gar nicht für ausgeschlossen halten, dass spätere Überlegungen zeigen werden, dass es in der Tat wünschenswert ist, die Rolle der SH-Ionen in diesem Falle und im allgemeinen die Rolle der verschiedenen Ionen bei den sonstigen Dissimilationsprozessen in den Vordergrund zu bringen.

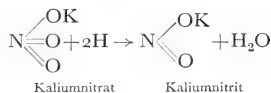
III. Der Denitrifikationsvorgang

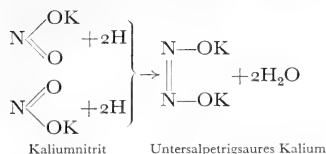
Bekanntlich wird der wichtige Denitrifikationsvorgang von sehr verschiedenen Bakterienarten bewirkt. Allen diesen Bakterien ist die Eigenschaft gemeinsam, dass sie Denitrifikation nur bewirken bei Anwesenheit organischer Substanzen,⁷² welche dabei oxydiert werden und zwar nur bei Abwesenheit freien Sauerstoffs. Ist Sauerstoff anwesend, dann bewirken sie dieselben Oxydationen mit Hilfe dieses Gases, jedoch bleibt das Nitrat in diesem Falle unangegriffen.

Es folgt nun ohne weiteres, dass wir die denitrifizierenden Bakterien als solche aerobe Organismen betrachten können, bei denen die Affinität des Protoplasmas zu Wasserstoff genügend abgeschwächt ist, um aus passenden Substraten eine Übertragung von Wasserstoff auch auf schwächere Akzeptoren als Sauerstoff, nämlich auf Nitrate zu ermöglichen.

Diese Vorstellung hat den Vorteil, dass sie bequem einen Einblick ermöglicht in die Stufenreaktionen der Umwandlung der Nitrate durch die denitrifizierenden Bakterien.

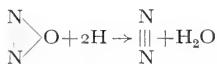
Man kann dabei die folgenden Teilreaktionen als wahrscheinlich annehmen:





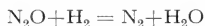
Dabei entstammt der für die Hydrierung benützte Wasserstoff der gleichzeitig anwesenden organischen Substanz, die nebenbei auch einer intramolekularen Dehydrierung (und Hydrierung) unterliegen kann, welche zu einer Kohlensäurebildung Anlass gibt. Die Kohlensäure wird aus dem untersalpetrigsauren Kalium die Säure in Freiheit setzen, die bekanntlich in verdünnter wässriger Lösung leicht das Anhydrid N_2O (Lachgas) abspaltet.

Das Anhydrid ist aber, wie auch aus den direkten Versuchen von Beijerinck und Minkman⁷³ hervorgeht, ebenfalls noch imstande als Wasserstoffakzeptor aufzutreten, wobei es in freien Stickstoff übergeführt wird:



Die gegebene Auffassung des Denitrifikationsprozesses erklärt also in deutlicher Weise die Entstehung von Nitriten und N_2O als Zwischenprodukte der Denitrifikation, wie dies von vielen Forschern, insbesondere auch von den beiden genannten, nachgewiesen worden ist. Dass verschiedene Bakterienarten die Denitrifikation bis zu verschiedenen Stufen bewirken, ist eine logische Konsequenz, denn die Nitrate, Nitrite und das Stickstoffoxydul sind als Wasserstoffakzeptoren nicht gleichwertig. Eine Wasserstoffaffinität, wie das Protoplasma einer bestimmten Bakterienart sie aufweist, kann gerade geeignet sein für die Wasserstoffübertragung aus einem speziellen Substrat auf Nitrate, nicht aber auf Nitrite oder auf Stickstoffoxydul.

Ein grosser Vorteil der gegebenen Anschauung besteht weiter darin, dass die ebenfalls von Beijerinck und Minkman festgestellte merkwürdige bakterielle Verarbeitung eines Gemisches von gasförmigem Wasserstoff und Stickstoffoxydul gemäss folgender Gleichung:



ganz in den Rahmen der üblichen Denitrifikationsprozesse hineinpasst.

Übrigens ist die besprochene Gruppe noch interessant in Hinsicht auf eine Bemerkung Wielands, folgendermassen formuliert: 'Die extremste Forderung der Dehydrierungstheorie, den Sauerstoff bei funktionellen Vorgängen der Zelle durch einen anderen Wasserstoffakzeptor zu ersetzen, hat sich bis jetzt nicht erfüllen lassen'.⁷⁴

Die denitrifizierenden Bakterien sind nun ein schönes Beispiel dafür, dass es aerobe Organismen gibt, bei denen sich der Luftsauerstoff vollständig durch einen anderen Wasserstoffakzeptor ersetzen lässt, ohne dass der normale Entwicklungsgang dieser Organismen davon beeinträchtigt wird.

IV. Dissimilationsprozesse der Bakterien der *Proteus*-Gruppe

Bekanntlich sind die Bakterien der *Proteus*-Gruppe einerseits denen der *Coli-aerogenes*-Gruppe nahe verwandt, indem bei beiden der anaerobe Zuckerabbau wesentlich in derselben Richtung verläuft. Andererseits unterscheidet die *Proteus*-Gruppe sich von der zweitgenannten dadurch, dass die Vertreter der ersteren unter *anaeroben* Bedingungen ebenfalls zu einer Verarbeitung von hydrolytischen Eiweisspaltprodukten imstande sind (Fäulnis). Der Chemismus dieser Vorgänge ist noch grösstenteils in Dunkel gehüllt. Durch Nawiasky⁷⁵ ist jedoch dieser Vorgang für den Fall des Asparagins als Substrat nahezu vollständig aufgeklärt.

Die von ihm aufgestellten Umlagerungen sind nun darauf zurückzuführen, dass ein Teil der aus Asparagin in erster Instanz durch Hydrolyse gebildeten Asparaginsäure vollständig bis zu Wasser, Kohlensäure und Ammoniak dehydriert wird, wobei ein anderer Teil des Asparagins als Wasserstoffakzeptor auftritt und dabei unter Ammoniakabspaltung zuerst zu Bernsteinsäure und teilweise weiter bis zu Essigsäure hydriert wird.

Der Unterschied zwischen den Dissimilationsprozessen der Koli-bakterien und denen der Proteusbakterien ist demnach darauf zurückzuführen, dass die Wasserstoffaffinität der letzteren noch beim $\text{pH} > 7$ die angegebene Wasserstoffübertragung ermöglicht.

Die vier gegebenen Beispiele liessen sich noch durch zahlreiche andere vermehren⁷⁶, doch glauben wir, dass sie genügen werden, um zu zeigen, auf welche mannigfache Art durch die katalytische Wasserstoffübertragungstheorie der Einblick in die Dissimilationsvorgänge vergrössert werden kann.

6. DIE ASSIMILATIONSVORGÄNGE IM LICHT DER
WASSERSTOFFÜBERTRAGUNGSTHEORIE

Wie schon in der Einleitung bemerkt wurde, lassen sich im Gesamtstoffwechsel vom physiologischen Standpunkte aus zweierlei Typen von Vorgängen unterscheiden: assimilatorische und dissimilatorische Prozesse. Hierbei sollen als dissimilatorische Vorgänge diejenigen bezeichnet werden, bei denen ein Teil der dargebotenen Nahrung in solche Produkte übergeführt wird, die von der Zelle entweder sofort oder doch bald ausgeschieden werden und dann in der Regel für sie keinen Wert mehr haben.⁷⁷ Diesen gegenüber stehen die assimilatorischen Vorgänge, also diejenigen chemischen Prozesse, welche die Nahrung in mehr oder weniger wichtige, doch normalerweise auftretende Zellsubstanzen überführen.

Während die Dissimilationsprozesse im Vorhergehenden genügend berücksichtigt sind, wollen wir jetzt die Frage kurz streifen, in wiefern auch die Bildung der Zellsubstanzen restlos – abgesehen von den hydrolytischen und esterifizierenden Vorgängen – auf eine Kette von katalytischen Wasserstoffübertragungen zurückgeführt werden kann.

Dabei ist zuerst zu berücksichtigen, dass, wie die Erfahrung lehrt, die assimilatorischen Vorgänge, die am deutlichsten bei der Zellvermehrung hervortreten, niemals stattfinden, ohne dass sich gleichzeitig dissimilatorische Vorgänge abspielen. Dieser Zusammenhang erklärt auch, dass bestimmte Assimilationsprodukte auf einem energetisch höheren Plan stehen als die verarbeitete Nahrung. Dies ist eben nur möglich, weil die freie Energie bei sonstigen Vorgängen eine grössere Abnahme gefunden hat, sodass auch die freie Energie des ganzen Systems abgenommen hat.

Der Vorgang der katalytischen Wasserstoffübertragung ist nun durchaus geeignet, eine nähere Vorstellung zu geben, wie dieser chemische Potentialhub einzelner Komponenten des ganzen Systems vor sich gehen kann. Denn es ist eben charakteristisch für diesen Vorgang, dass bei diesem Prozess der Wasserstoffakzeptor energetisch gehoben wird, dem dann gegenüber steht, dass das Substrat bei der Dehydrierung einen grösseren Verlust an freier Energie erfährt. In diesem Zusammenhange sei bemerkt, dass auch Wieland betont, dass nur solche Reaktionen im Spiel der Hydrierungen und Dehydrierungen eintreten, bei denen der Energiegewinn bei der Hydrierung grösser ist als

die Aufwendung zur Dehydrierung. Man kann diesen Satz auch so formulieren: 'dass nur solche Reaktionen eintreten, bei denen die Zunahme der freien Energie, welche der Akzeptor bei der Hydrierung erfährt, kleiner ist als die Abnahme der freien Energie, welche das Substrat bei der Dehydrierung erfährt'.

Aus dieser Auseinandersetzung folgt einerseits, dass der chemische Potentialhub einzelner Reaktionsprodukte durchaus nicht auf assimilatorische Vorgänge beschränkt ist, sondern dass dieser vielmehr auch bei dissimilatorischen Vorgängen öfters auftritt. Daraus können wir schliessen, dass in physikochemischer Hinsicht durchaus kein Unterschied zwischen beiden Typen von chemischen Prozessen existiert, so dass von vornherein sich nichts dagegen widersetzt, auch die Entstehung der typischen Assimilationsprodukte auf eine Kette katalytischer Wasserstoffübertragungen zurückzuführen.⁷⁸

Im folgenden werden wir uns darauf beschränken, das Problem für die wichtigsten der verschiedenen Substanzen, welchen man in den Zellen begegnet, nämlich die Kohlenhydrate, Fette und Eiweissstoffe, kurz zu betrachten.

Was zuerst die Kohlenhydrate betrifft, denen man als Reservesubstanz und auch als Gerüstsubstanz häufig begegnet, so kann folgendes bemerkt werden: soweit die zu verarbeitende Nahrung schon Kohlenhydrate enthält, kann die Bildung der komplexen Kohlenhydrate einfach durch Kondensation der einfachsten Bausteine, nämlich der Zucker, stattfinden. Ausgeschlossen ist es dabei jedoch nicht, dass sich die Sache nicht so einfach verhält, und dass die Synthese ihren Ausgangspunkt nimmt bei irgendwelchen Zucker-Abbauprodukten der Dissimilationsprozesse. Dabei kann man sich denken, dass irgendein dehydriertes Substrat unter Umständen wieder als Wasserstoffakzeptor fungiert bei irgend einer Dehydrierung, welche mit grösserer Abnahme an freier Energie verläuft als die Dehydrierung des ursprünglichen Substrates.

Dieselbe Situation findet man dann zurück in den Fällen, worin die Nahrung nur Substanzen enthält, welche in energetischer Hinsicht minderwertiger sind als die zu synthetisierenden Kohlenhydrate, wie dies z. B. zutrifft für die Rückverwandlung von Milchsäure in Glykogen in den Muskelzellen bei der aeroben Phase der Muskelarbeit. Auch hier brauchen offenbar nur die bei der Bildung der Milchsäure eingetretenen Dehydrierungen und Hydrierungen rückgängig

gemacht zu werden, was durch die gleichzeitig stattfindende Verbrennung eines Teiles der Milchsäure energetisch ermöglicht wird. Zwar wollen wir hier offen gestehen, dass es nicht ohne weiteres verständlich ist, wie sich dies Rückgängigmachen bei den *intra*-molekularen Dehydrierungen und Hydrierungen abspielt, aber immerhin kann man sich denken, dass hier die entwertete Atomgruppe zuerst energetisch gehoben wird, indem sie als Akzeptor bei einer 'Dissimilationsdehydrierung' auftritt und dann die zweite, ursprünglich energetisch gehobene Atomgruppe in einem neuen Dehydrierungsvorgang wieder degradiert wird.

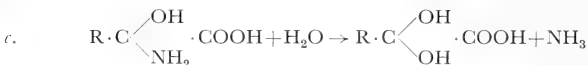
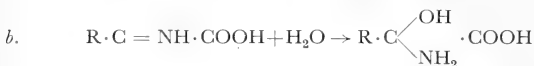
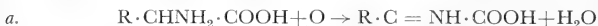
Auch in den Fällen, worin die Kohlenhydratsynthese aus Kohlensäure stattfindet, unterliegt es kaum einem Zweifel, dass hierbei an erster Stelle eine Reduktion dieser Säure über Ameisensäure zu Formaldehyd stattfindet. Wir werden hierbei die photochemische Kohlensäureassimilation durch die grünen Pflanzen ausser Betracht lassen,⁷⁹ obgleich der enge Zusammenhang dieses Vorganges mit der Kohlensäureassimilation durch die autotrophen Bakterien unverkennbar ist. Es ist nun zweifellos kein Zufall, dass wir das Vermögen zur Kohlensäureassimilation unter den Bakterien nur bei denjenigen Gruppen antreffen, die zu der schwierigen Dehydrierung anorganischer Substrate imstande sind. In unserem Vorstellungskreis sind diese Bakterien – vor allem die nitrifizierenden- und Schwefelbakterien – dazu befähigt, weil die grosse freie Affinität ihres Protoplasmas zu Wasserstoff es ermöglicht, die Wasserstoffatome in Substraten, worin dieselben fest gebunden sind, zu aktivieren. Die Kohlensäure (in ihrer Hydratform) wird aber von der exzeptionell grossen Affinität des Protoplasmas zu Wasserstoff aktiviert werden, wodurch sie befähigt wird als Akzeptor aufzutreten.

Was an zweiter Stelle die Fette betrifft, so können wir uns darüber ganz kurz fassen, weil diese Frage eine weitgehende Lösung gefunden hat durch die Untersuchungen von Hahn und Kinttoff⁸⁰. Ohne näher darauf einzugehen, wollen wir nur konstatieren, dass die Vorstellung viel Wahrscheinliches hat, dass die Fettsynthese bei dem Azetaldehyd ihren Ausgangspunkt nimmt und aus dieser Substanz durch abwechselnde Kondensation (d. h. intermolekulare Wasserstoffübertragung) und Hydrierung allmählich in die höheren Fettsäuren umgewandelt wird. Der Wasserstoff – und damit die benötigte Energie – für diese Hydrierung wird dabei den Dehydrierungen der gleich-

zeitig verlaufenden Dissimilation eines anderen Teiles des Nährsubstrates entstammen.

Schliesslich möchten wir noch einige Aufmerksamkeit der Synthese des Eiweisses schenken. Durch die rezente Arbeit von Knoop und Oesterlin⁸¹ ist dieser Vorgang, jedenfalls was die Synthese der Aminosäuren betrifft, klargestellt. Die genannten Forscher konnten schöne experimentelle Nachweise dafür bringen, dass diese Synthese der Hauptsache nach den umgekehrten Weg einschlägt, welcher bei der oxydativen Dissimilation der Aminosäuren befolgt wird.

Diese oxydative Verarbeitung der Aminosäuren ist eine der verbreitetsten Dissimilationsprozesse. Wahrscheinlich spielen sich dabei die folgenden Reaktionen ab:



Die gebildete Ketosäure wird dann weiter in der bekannten Weise dehydriert, wobei als erstes Zwischenprodukt zweifellos der Aldehyd



entstehen wird (Streckersche Reaktion).

Auch dieser Dissimilationsprozess ist also auf eine Kombination von Hydrolysen und katalytischen Wasserstoffübertragungen zurückzuführen.

Die wichtigen Ergebnisse von Knoop und Oesterlin machen es nun äusserst wahrscheinlich, dass die Synthese der Aminosäuren umgekehrt ihren Ausgangspunkt nimmt bei den Ketosäuren und Ammoniak, welches System dann durch eine bei der Dissimilation stattfindende Dehydrierung (mit grösserem Potentialfall als die der Dehydrierung von Aminosäure zu Iminosäure) zu Aminosäure hydriert wird.

Auf die überaus grosse Wahrscheinlichkeit, dass weiter die Organismen, welche zur Eiweissynthese aus atmosphärischem Stickstoff im-

stande sind, als erste Stufe den freien Stickstoff zu Ammoniak hydrieren mit Hilfe des dem Dissimilationssubstrat entstammenden aktivierten Wasserstoffs, ist schon von Wieland hingewiesen worden ('biologische Habersynthese'). Dabei ist es jedoch nicht ausgeschlossen, dass diese Hydrierung nur stattfindet, wenn der Stickstoff zuvor ebenfalls aktiviert wird, genau wie dies beim Sauerstoff mit Hilfe der Warburgschen Eisenverbindung immer geschieht. Der eine von uns (D.) hat die grosse Bedeutung eines Zusatzes kolloidaler Eisenverbindungen für die Entwicklung der anaeroben stickstoffbindenden Bakterien (*Clostridium pasteurianum* Winogradsky) mit Sicherheit feststellen können, und auch für die wichtigsten aeroben stickstoffbindenden Bakterien (*Azotobacter chroococcum* Beijerinck) ist ähnliches schon früher von Krzemieniewski und von Prazmowski nachgewiesen.

Obleich die hier gegebene Übersicht äusserst unvollständig ist und verschiedene Punkte zweifellos weiterer Aufklärung bedürfen, glauben wir doch, dass sie genügen wird den Eindruck zu erwecken, dass das Prinzip der katalytischen Wasserstoffübertragung geeignet ist, auch die assimilatorischen Vorgänge in ihren Hauptzügen verständlich zu machen.

Die gegebene Vorstellung vom Wesen der assimilatorischen Vorgänge erklärt auch den unverkennbaren Zusammenhang zwischen der Natur der Dissimilationsprozesse der verschiedenen Organismengruppen und der Fähigkeit dieser Organismen zu assimilatorischen Synthesen. Dies zeigt sich z. B. in der Tatsache, dass die wahren Milchsäurebakterien, welche durch einen Mangel von sowohl kräftiger Wasserstoff- als Sauerstoffaffinität gekennzeichnet sind, nicht imstande sind, mit anorganischen Stickstoffverbindungen oder mit Verbindungen wie Harnstoff auszukommen. Auch das ganze vielumstrittene Biosproblem lässt sich mit grosser Wahrscheinlichkeit auf denselben Umstand zurückführen.

7. ÜBERBLICK ÜBER DIE AUFGESTELLTE THEORIE DER KATALYTISCHEN WASSERSTOFFÜBERTRAGUNG ALS KERN DES BIOCHEMISCHEN GESCHEHENS

Die in den vorhergehenden Teilen niedergelegten Ausführungen kann man kurz zusammenfassen als einen Versuch, die durch Wieland für die oxydativen Dissimilationsprozesse ausgearbeitete Dehydrierungs-

theorie konsequent auch auf die fermentativen Dissimilationsprozesse zu übertragen. Obgleich die Wielandsche Theorie früher schon mehrmals für bestimmte Teilreaktionen in den fermentativen Dissimilationsprozessen zur Erklärung herangezogen worden war, stand eine konsequente Durchführung dieses Prinzips auf alle – oder jedenfalls auf die wichtigsten – Dissimilationsprozesse noch aus.

Der gemachte Versuch scheint uns in hohem Grade erfolgreich, indem man, sich zuerst beschränkend auf die Prozesse, wobei Zucker (Glukose) als Dissimilationssubstrat fungiert, klar erblicken kann, wie sich die verschiedensten bekannten Dissimilationsprodukte mit Hilfe einer Kette von katalytischen Wasserstoffübertragungsreaktionen von dem Substrat ableiten. Die von Wieland verteidigte Auffassung der Wasserstoffaktivierung, die für die aeroben Dissimilationsprozesse mehr oder weniger eine wissenschaftliche Abstraktion ist, sieht man allmählich bei den mehr anaeroben Organismen zur Wirklichkeit werden, indem hier grosse Quantitäten gasförmigen Wasserstoffs aus dem Gärungsgefäß entweichen. Sonnenklar tritt weiter der allmähliche Übergang des aeroben Lebens in das anaerobe an den Tag.

Es tritt nicht nur bei der obigen Betrachtung die volle Berechtigung des berühmten Pasteurschen Satzes 'La fermentation est la vie sans air' sehr deutlich hervor, sondern die Ähnlichkeit im Wesen der Atmung und Gärung wird so vollkommen, dass man mit gleichem Rechte die Atmung als eine von dem – durch Warburgs Eisenverbindung aktivierten – Sauerstoff in andere Bahnen gelenkte Gärung auffassen kann.⁸² Die nur nebensächliche Bedeutung des Sauerstoffs tritt besonders schön bei den denitrifizierenden Bakterien hervor, bei denen wir den Fall realisiert finden, dass der Sauerstoff sich ohne irgend einen nachteiligen Einfluss auf den normalen Entwicklungsgang durch andere Akzeptoren (Nitrate) ersetzen lässt.

Neben der gegebenen Erweiterung der Wielandschen Theorie in quantitativer Hinsicht haben wir die Theorie der Oxydoreduktion auch in prinzipieller Weise erweitert, indem wir darauf hinwiesen, dass es möglich ist, auch diejenigen Teilreaktionen, welche auf einer einfachen Umlagerung bestimmter Substanzen beruhen, als katalytische Wasserstoffübertragungsprozesse *intramolekularer* Art zu betrachten. Dasselbe gilt ebenfalls für die im Stoffwechsel nicht seltenen Kondensationsreaktionen.

Dadurch war die Möglichkeit gegeben, *alle* von einer Zelle be-

wirkten Teilprozesse der Dissimilation – abgesehen von den hydrolytischen und esterifizierenden Vorgängen, welche aber grösstenteils nur vorbereitender Natur sind – der Wirkung eines einzelnen Agens zuzuschreiben.

Es braucht kaum betont zu werden, wie die Vorstellung der biochemischen Arbeitsweise der lebenden Zelle hierdurch vereinfacht wird. Bisjetzt ist es fast allgemein üblich, jede der in dem Dissimilationsprozess sichergestellten Teilreaktionen der Wirkung eines spezifischen Enzyms zuzuschreiben. Diese Enzyme haben dann je nach der Natur der von ihnen bewirkten Reaktionen Namen bekommen als: Katalase, Reduktase, Zymase, Laktozymase, Alkoholoxydase, Karboxylase, Karboligase, Methylglyoxalase, Aldehydmutase, Schardingers Enzym u. s. w.

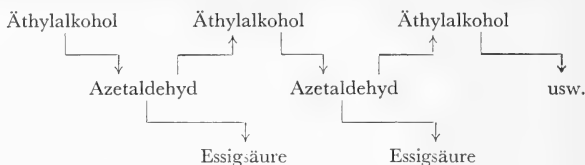
Auf Grund des Vermögens einer Zelle, Bernsteinsäure zu Fumar-säure zu dehydrieren, wurde z. B. auf die Anwesenheit einer Sukzino-fumarase geschlossen. So lange man seine Aufmerksamkeit nur auf die normalerweise vorkommenden Stoffwechselvorgänge lenkte, war eine derartige Handlungsweise noch durchführbar; seitdem aber die moderne Forschung jeden Tag deutlicher zeigt, dass die Zellen auch gegenüber ihnen in der Natur nie oder äusserst selten gebotenen Substanzen kräftige Wirkungen entfalten, führt diese Auffassung ad absurdum. Man kann sich doch unmöglich vorstellen, dass, um nur ein Beispiel zu geben, die von Beijerinck beschriebenen Bakterien, welche Querzit in Pyrogallol überführen, dies bewirken dank der Anwesenheit einer Querzitopyrogallolase, welche sie für den seltenen Fall, dass sie Querzit begegnen, bereit halten!

Obleich man in beschränktem Masse schon gefühlt hatte, dass die Wirkung der genannten Enzyme nicht so streng spezifisch aufgefasst werden konnte und man z. B. der Karboxylase eine allgemeine Wirkung den α -Ketosäuren gegenüber zuschrieb, ist es nicht schwierig, an einem Beispiel zu demonstrieren, welche Befreiung die von uns angegebene weitgehende Unifikation der wirksamen Agentia mit sich bringt.

Durch Neuberg und Windisch⁸³ ist in einer schönen experimentellen Untersuchung gezeigt worden, dass Essigbakterien beim Abschluss freien Sauerstoffs Aldehyde weitgehend umlagern in gleiche Teile der betreffenden Alkohole und Säuren (z. B. Azetaldehyd in Äthylalkohol und Essigsäure). Die genannten Forscher schliessen

daraus auf die Anwesenheit eines die Dismutation des Aldehyds bewirkenden Enzyms in diesen Bakterien, wogegen wenig einzuwenden scheint. Dies führt sie jedoch zu dem Schluss, dass ein Essigbakterium, welches unter normalem Sauerstoffzutritt Alkohol oxydiert und dabei zweifellos als erstes Oxydationsprodukt Azetaldehyd bildet, diesen Aldehyd auch unter aeroben Verhältnissen mit Hilfe des genannten Enzyms nur zur Hälfte in Essigsäure überführen und die zweite Hälfte in Alkohol rückverwandeln wird, um denselben wieder in der angegebenen Weise zu verarbeiten.

So entsteht Neubergs 'Pilgerschrittschema' für die normale durch Essigbakterien bewirkte Oxydation von Alkohol zu Essigsäure:



Diese an sich wenig wahrscheinliche Vorstellung begründen sie weiter durch einen einzigen Versuch, bei dem mit einem Trockenpräparat der Bakterien auch bei einem offenbar sehr mangelhaften Luftzutritt ein Teil des verarbeiteten Azetaldehyds als Alkohol wiedergefunden werden konnte.

Die Notwendigkeit zur Annahme eines derartig komplizierten Vorganges fällt sofort weg, wenn wir die Dismutierung des Aldehyds unter *anaeroben* Bedingungen der Wirkung des allgemeinen Wasserstoff übertragenden Agens zuschreiben, welches bei Abwesenheit eines anderen Akzeptors den der Dehydrierung eines Moleküls Azetaldehyd entstammenden Wasserstoff auf ein zweites Molekül überträgt. Wir sehen dann sofort ein, dass bei *tadelloser Lüftung* der aus Äthylalkohol primär gebildete Azetaldehyd normalerweise ebenfalls einer Dehydrierung unterliegen wird, aber dass in diesem Falle der aktivierte Sauerstoff, nicht ein zweites Molekül Aldehyd, als Akzeptor für diesen Wasserstoff auftreten wird. Eine Rückbildung von Alkohol wird dabei höchstens von ungenügend belüfteten Zellen bewirkt werden.

Man wird gegen unsere Auffassung der einheitlichen Natur der wirksamen Agentia den Einwand erheben, dass demnach alle Zellen zu allen diesen Umsetzungen imstande sein müssten. Dagegen wollen

wir zunächst bemerken, dass die moderne Forschung auch immer deutlicher lehrt, dass die verschiedensten Zellen zu überraschend vielen Umsetzungen fähig sind. Es erübrigt sich wohl, hierfür Beispiele zu geben, da sie in grösster Menge zu Gebote stehen.

Dass jedoch das Agens verschieden gearteter Zellen nicht dasselbe ist und bestimmte Fälle katalytischer Wasserstoffübertragung merklich bevorzugt, andere nur langsam oder mit praktisch unmerklicher Geschwindigkeit fördert, findet seine Ursache in einer *quantitativen* Abstimmung des Katalysators.

Im Anschluss an frühere Betrachtungen über den Chemismus der Katalyse bei rein chemischen Prozessen haben wir nun die nachfolgende Hypothese über die Ursache der katalytischen Wasserstoffübertragung aufgestellt. Die katalytische Wirkung des Agens – welches wir vorläufig einfach mit dem Protoplasma der Zelle identifiziert haben – sollte bedingt sein durch die freie Affinität des Protoplasmas zu Wasserstoff oder zu Sauerstoff. Diese Affinität würde für eine jede artspezifische Zelle eine bestimmte Grösse haben und eben diese Grösse, welche für bestimmte Katalysen optimal sein würde, würde die Richtung der Dissimilation bestimmen. Die verschiedenen Zellen würden sich je nach dem Masse ihrer Aerobie und Anaerobie gewissermassen in eine Reihe ordnen lassen mit abnehmender Wasserstoffaffinität, welche bei den Anaerobiern in eine zunehmende Sauerstoffaffinität umschlägt. Diese Hypothese wird gestützt durch die Tatsache, dass sie einen Zusammenhang gründet mit anderen hervortretenden Eigenschaften der betrachteten Zellen, vor allem mit der Sauerstoffempfindlichkeit der anaeroben Organismen und mit dem Vermögen der Verarbeitung gasförmigen Wasserstoffs durch viele ausgesprochen aerobe Organismen. Auch die Empfindlichkeit der am kräftigsten oxydierenden Organismen, der nitrifizierenden Bakterien, gegenüber den für die meisten übrigen Zellen am besten assimilierbaren organischen Substanzen, lässt sich in diesem Rahmen betrachten.

Die gegebene Hypothese musste jedoch noch eine sehr plausible Erweiterung erfahren, indem angenommen werden musste, dass die genannte Wasserstoffaffinität der Zelle keine absolut konstante Grösse ist, sondern dass dieselbe innerhalb des Gebietes der für die Zelle zulässigen Wasserstoffionenkonzentrationen mit dieser Konzentration schwankt. Die unendliche Verschiedenheit des biochemischen Ver-

haltens der verschiedenen artspezifischen Zellen wäre dabei zurückzuführen auf eine Verschiedenheit einer einzelnen Eigenschaft des Protoplasmas – der Wasserstoff- bzw. der Sauerstoffaffinität – in ihrer Abhängigkeit von den, für jede Art typischen, zulässigen Wasserstoffionenkonzentrationen. Die Tatsache, dass auch die Dehydrierungsneigung der Substrate und Hydrierungsneigung der Akzeptoren bekanntlich eine Funktion der Wasserstoffionenkonzentrationen sind, trägt wesentlich dazu bei, die äussere Verschiedenheit des biochemischen Geschehens trotz seiner inneren Einheit zu vermehren.

Wenn man daneben berücksichtigt, dass die oxydative oder fermentative Dissimilation von Zuckerarten immer zur intermediären oder definitiven Bildung von Säuren, die Dissimilation der hydrolytischen Eiweisspaltprodukte immer zur Bildung von Ammoniak führt, dann kommt man zu der Ansicht, die Anpassung der verschiedenen Zellen an diese wichtigsten Substratgruppen darin zu begründen, dass das Protoplasma dieser Zellen bei den dazu gehörenden Wasserstoffionenkonzentrationen eine passende Wasserstoffaffinität erworben hat.

Schliesslich haben wir gemeint andeuten zu können, dass nicht nur die dissimilatorischen, sondern auch die assimilatorischen Vorgänge wesentlich auf katalytische Wasserstoffübertragung zurückgeführt werden können.

8. SCHLUSSBETRACHTUNG

Wir möchten diese Arbeit nicht abschliessen, ohne klar auszusprechen, dass wir uns vollbewusst sind, dass hier nur ein sehr unvollendeter Versuch gemacht worden ist, eine gewisse Ordnung in die biochemischen Vorgänge zu bringen. Der Umfang des betrachteten Materials liess es nicht zu, die Einzelheiten immer genügend zu begründen. Wir sind also darauf vorbereitet, dass der kritische Leser an vielen Stellen Einwände erheben wird. Doch sind wir überzeugt, dass die Anwendung der gegebenen Betrachtungen als Arbeitshypothese in vielen Spezialfällen Vorteile abwerfen wird.

Immerhin sind wir völlig überzeugt, dass es notwendig sein wird, die angewendeten Begriffe auf eine festere, physikochemische Basis zu stellen. Dabei unterliegt es kaum einem Zweifel, dass die von uns benutzte freie Affinität des Protoplasmas zu Wasserstoff oder zu Sauerstoff ihren quantitativen Ausdruck finden wird in dem Oxydoreduk-

tionspotential des Protoplasmas. Angesichts der Tatsache, dass in jüngster Zeit ein erfolgreicher Versuch gemacht worden ist, dieses Potential für das Protoplasma einer lebenden Zelle zu bestimmen,⁸⁴ öffnet sich die Möglichkeit, zahlenmässig zu entscheiden, ob eine Zelle imstande sein wird, eine bestimmte gekoppelte Dehydrierung und Hydrierung zu bewirken. Wenn ausserdem einmal die Oxydoreduktionspotentiale für die Systeme Substrat-dehydriertes Substrat und Akzeptor-hydrierter Akzeptor in genügenden Fällen festgestellt worden sind, wird man auch dazu kommen können vorauszusagen, ob ein bestimmtes einfaches Substanzsystem für die betreffende Zelle eine passende Nahrung bieten wird oder nicht.

In diesem Zusammenhang wird auch das nähere Studium der anorganischen wasserstoffübertragenden Katalysatoren zweifellos wichtige Anhaltspunkte liefern können. Die Wielandschen Versuche lehren, dass das Palladium in seinen oxydoreduktiven Eigenschaften dem Protoplasma vieler aeroben Bakterien (u.m. Essigbakterien) sehr nahe steht.

Obgleich eine typische Vergärung des Zuckers mit Hilfe anorganischer Katalysatoren wohl ausgeschlossen sein wird, da bei den biologischen Vergärungen die Bildung und Spaltung der Phosphorsäureester unentbehrlich scheint, werden die rein oxydoreduktiven Teilprozesse sich zweifellos alle mit anorganischen Katalysatoren reproduzieren lassen. Dies ist ja auch heute schon weitgehend der Fall.

Wie dies auch sein möge, fest steht wohl, dass die mit Rücksicht auf die aeroben Atmungsprozesse gemachte Aussage Thunbergs: 'dass der Wasserstoff als das elementare, gemeinsame Brennmaterial der Zellen zu betrachten ist' in seiner Tendenz noch viel zu beschränkt ist, und dass in den kommenden Jahrzehnten der Wasserstoff in seinen verschiedenen Aktivierungsstadien im Mittelpunkt der ganzen biochemischen Forschung stehen wird.

NOTEN UND LITERATUR

1. C. OPPENHEIMER, Die Fermente und ihre Wirkungen, 5 Aufl., Leipzig 1926.
2. C. NEUBERG und C. OPPENHEIMER, Biochem. Z. 166, 450, 1925.
3. a.a.O., S. 1213.
4. In Übereinstimmung hiermit ist die in deutscher Sprache mehr übliche Unterscheidung in Betriebsstoffwechsel und Baustoffwechsel.
5. So z.B.: J. H. QUASTEL, Biochem. J. 18, 365, 1925; C. NEUBERG und F. WINDISCH, Biochem. Z. 166, 454, 1925.
6. Vergleiche z.B.: O. MEYERHOF, Naturwissenschaften 7, 253, 1919; Ders., Ber. deutsch. chem. Ges. 58, 991, 1925; S. KOSTYTSCHEW, Z.f. physiol. Chem. 111, 141, 1920; M. SCHOEN, Bull. de l'Inst. Pasteur 24, 1, 1926. — Eine nähere experimentelle Begründung lieferten F. HAYDUCK und H. HAEHN, Biochem. Z. 128, 586, 1922.
7. Dass man heute das Adjektiv 'fermentativ' noch öfters gebraucht als Synonym für 'enzymatisch', bringt keine Änderung in unsere Anschauung. Dass man eben dazu übergegangen ist, die Begriffe 'Enzym' und 'enzymatisch' einzuführen, beweist schon, dass man gefühlt hat, dass die älteren Begriffe 'Ferment' und 'fermentativ' in genannter Hinsicht verwirrend wirken.
8. Es erübrigt sich wohl, hier die betreffende umfangreiche Literatur vollständig anzuführen. Es möge nur verwiesen werden auf die Übersicht in Oppenheimer, 'Die Fermente', und weiter auf die folgenden zusammenfassenden Darstellungen von WIELAND selbst: 'Ergebnisse der Physiologie' 20, 477, 1922; Ber. deutsch. chem. Ges. 55, 639, 1922 und auf das von WIELAND bearbeitete Kapitel in Oppenheimers 'Handbuch der Biochemie', 2. Aufl., Bd. II, S. 252, 1923.
9. W. PALLADIN, Biochem. Z. 18, 151, 1909; Id. 27, 442, 1910; Id. 35, 1, 1911; Id. 49, 381, 1913; Id. 60, 171, 1914.
10. G. BREDIG und F. SOMMER, Z. f. physik. Chem. 70, 34, 1910; G. BREDIG, Ber. deutsch. chem. Ges. 47, 546, 1914.
11. Für die Warburgschen Ansichten vergleiche man seine diesbezüglichen Abhandlungen in Biochem. Z. 119, 1921 bis heute.
12. Herr Professor Oppenheimer war so freundlich zu berichten, dass er unsere Separata erst empfangen hatte, als die betreffenden Abschnitte schon im Druck fertig standen.
13. OPPENHEIMER, a.a.O., S. 1214.
14. Wie bei der biochemischen Phosphorylierung vorangehend an den Zuckerzerfall; vergl. A. J. KLUYVER und A. P. STRUYK, Versl. Kon. Akad. v. Wet. Amsterdam 35, 177, 1926.
15. A. HARDEN, J. Chem. Soc. 79, 612, 1901.
16. Beispiele hierfür findet man u.a. bei A. Kirow, Untersuchungen zur Butter-säuregärung, Ref. Centr. f. Bakt. Parasitenk. II. Abt., 31, 534, 1912.
17. T. BAUMGÄRTEL, Grundriss der theoretischen Bakteriologie, Berlin 1924.
18. E. C. GREY, Proc. Royal Soc. Ser. B. 87, 472, 1914; siehe auch E. C. GREY, Ibid. 90, 92, 1919.
19. Es erübrigt sich hier wohl, eine vollständige Literaturangabe dieser allgemein

bekannten Abhandlungen zu geben. Bis 1923 findet man sie in übersichtlicher Weise wiedergegeben bei A. Harden, *Alcoholic Fermentation*, 3rd Ed., London 1923.

20. Für diese Ansicht war auch früher Kostytschew eingetreten. Vergleiche S. KOSTYTSCHEW, *Z. f. physiol. Chem.* 79, 130, 1912; *Ibid.* 79, 359, 1912.
21. C. NEUBERG und F. F. NORD, *Biochem. Z.* 96, 133, 1919; C. NEUBERG, F. F. NORD und E. WOLFF, *Ibid.* 112, 144, 1920; C. NEUBERG und B. ARNSTEIN, *Ibid.* 117, 269, 1921; H. KUMAGAWA, *Ibid.* 131, 157, 1922. Ein Vorläufer ist E. C. GREY, der 1913 schon die Bildung von Azetaldehyd bei der von *B. coli* bewirkten Gärung nachwies (*Biochem. J.* 7, 359, 1913).
22. W. H. PETERSON and E. B. FRED, *J. Biol. Chem.* 44, 29, 1920.
23. W. C. DE GRAAFF und A. J. LE FÈVRE, *Biochem. Z.* 155, 313, 1925.
24. Ausnahmsweise wird auch Fructose als Substrat berücksichtigt werden.
25. Vergleiche hierzu die folgenden Arbeiten über den Chemismus der oxydativen Verarbeitung von Zucker durch Schimmelpilze: J. N. CURRIE, *J. Biol. Chem.* 31, 15, 1917. M. MOLLIARD, *C.R. Soc. Biol.* 82, 719, 1919; *Ibid.* 83, 479, 1920; *Ibid.* 87, 967, 1922; *Ibid.* 90, 1395, 1924; *C. R. Acad. d. Sci.* 174, 881, 1922. W. BUTKEWITSCH, *Biochem. Z.* 129, 445, 1922; *Ibid.* 129, 455, 1922; *Ibid.* 129, 464, 1922; *Ibid.* 131, 327, 1922; *Ibid.* 131, 338, 1922; *Ibid.* 132, 556, 1922; *Ibid.* 136, 224, 1923; *Ibid.* 142, 195, 1923; *Ibid.* 154, 177, 1924. Idem, *Jahrb. f. wissensch. Botanik* 64, 637, 1925. R. FALCK und VAN BEYMA THOE KINGMA, *Ber. deutsch. chem. Ges.* 57, 915, 1924. R. FALCK und S. W. KAPUR, *Ibid.* 57, 920, 1924. K. BERNHAUER, *Biochem. Z.* 153, 517, 1924. C. WEHMER, *Ber. deutsch. chem. Ges.* 58, 2616, 1925. R. SCHREYER, *Ber. deutsch. chem. Ges.* 58, 2647, 1925.
Für den weiteren Abbau des Zuckers über das Glukonsäurestadium hinaus durch Essigbakterien verweisen wir nach: A. J. KLUYVER und F. J. G. DE LEEUW, *Die deutsche Essigindustrie* 29, 175, 1925; und F. VISSER 'T HOOFT, *Biochemische onderzoekingen over het geslacht Acetobacter*, Diss. Delft 1925.
26. A. HARDEN and W. J. YOUNG, *Proc. Royal Soc. Ser. B.* 77, 405, 1906.
27. G. EMBDEN und F. LAQUER, *Z. physiol. Chem.* 93, 94, 1914; *Ibid.* 98, 181, 1917; *Ibid.* 113, 1, 1921.
28. A. I. VIRTANEN, *Z. physiol. Chem.* 138, 136, 1924.
29. A. I. VIRTANEN, *Comment. physico-mathematicae Societas Scientiarum Fennica* II, Nr. 20, 1925.
30. In seiner zuletzt zitierten Abhandlung hat Virtanen jedoch wahrscheinlich gemacht, dass in den Fällen, in denen die normalerweise stattfindende biochemische Phosphorylierung durch Mangel an Ko-Enzym nicht eintreten kann, bei den Propionsäurebakterien auch eine direkte Spaltung des Glukosemoleküls in Verbindungen der C₂- und der C₄-Reihe auftritt. Diese Spaltung sollte nach Virtanen auch im normalen Stoffwechsel der genannten Bakterien als Nebenprozess stattfinden.
31. A. J. KLUYVER und A. P. STRUYK, a.a.O. Für die Dokumentierung der oben gegebenen Auffassung möge auf diese Abhandlung verwiesen werden.
32. J. H. NORTHROP, L. H. ASHE and R. R. MORGAN, *J. Ind. Eng. Chem.* 11, 723,

1919. J. H. NORTHROP, L. H. ASHE and K. J. SENIOR, *J. Biol. Chem.* **39**, 1, 1919.
- C. F. ARZBERGER, W. H. PETERSON and E. B. FRED, *J. Biol. Chem.* **44**, 465, 1920.
- W. H. PETERSON, E. B. FRED and J. H. VERHULST, *J. Ind. Eng. Chem.* **13**, 757, 1921.
- E. B. FRED, W. H. PETERSON and J. A. ANDERSON, *Ibid.* **15**, 126, 1923.
33. Vorläufige Publikation in: *Tijdschr. v. Vergl. Geneesk.* 1924. Ausführliche Publikation erscheint demnächst.
34. A. HARDEN, *J. Chem. Soc.* **79**, 612, 1901. A. HARDEN and D. NORRIS, *Proc. Royal Soc. Ser. B.* **84**, 492, 1912.
35. E. C. GREY, *Proc. Royal Soc. Ser. B.* **87**, 461, 1914; *Ibid.* **87**, 472, 1914; *Ibid.* **90**, 75, 1919; *Ibid.* **90**, 92, 1919; *Ibid.* **91**, 294, 1920.
36. JAN SMIT, *Bacteriologische en chemische onderzoekingen over de melkzuurgisting*, Diss. Amsterdam 1913.
37. W. H. PETERSON and E. B. FRED, *J. Biol. Chem.* **41**, 431, 1920; *Ibid.* **42**, 273, 1920; *Ibid.* **42**, 175, 1920; *Ibid.* **44**, 29, 1920; *Ibid.* **48**, 385, 1921; *Ibid.* **53**, 111, 1922; *Ibid.* **64**, 643, 1925.
38. A. I. VIRTANEN. *Societas Scientiarum Fennica, Comment. Physico-Mathematicae* **1**, Nr. 36; 1923; *Ibid.* **2**, Nr. 20; 1925. Siehe auch: J. M. SHERMAN, *J. Bact.* **6**, 379, 1921.
39. A. I. VIRTANEN, a.a.O.
40. Ausser verschiedenen technisch wichtigen Publikationen sind hier zu erwähnen H. B. SPEAKMAN, *J. Biol. Chem.* **41**, 319, 1920; *Ibid.* **43**, 401, 1920; *Ibid.* **58**, 395, 1923; H. B. SPEAKMAN and J. F. PHILIPS, *J. Bact.* **9**, 183, 1924; J. REILLY, W. J. HICKINBOTTOM, FR. R. HENLEY and A. C. THAYSEN, *Biochem. J.* **14**, 229, 1920; W. H. PETERSON, E. B. FRED and L. G. SCHMIDT, *J. Biol. Chem.* **60**, 627, 1924.
41. Ausführliche Publikation erscheint demnächst.
42. Nur die hydrolytischen Vorgänge und Esterifikationen fallen – wie schon mehrmals bemerkt – aus diesem Schema. Bei der Zuckerdissimilation begegnen uns nur die Bildung der Hexosemonophosphorsäureester und die damit zusammenhängende Spaltung der Triosemonophosphorsäureester.
43. E. F. ARMSTRONG and T. P. HILDITCH, *Proc. Royal Soc. Ser. A.* **96**, 322, 1920. Vergl. auch N. ZELINSKY und N. GLINKA, *Ber.* **44**, 2305, 1911.
44. Eine Übersicht von Böesekens 'Dislokationstheorie' findet man in: *Rec. trav. chim.* **39**, 623, 1920; und *Proc. Royal Acad. Amsterdam* **25**, 210, 1922.
45. Die Grundlagen der für unsere Betrachtungen ebenfalls sehr wichtigen Anschauungen von Prins findet man in seiner Dissertation 'Bijdrage tot de kennis der katalyse', Delft 1912. Speziell dürfte auch Erwähnung finden seine Abhandlung über den Zusammenhang zwischen Katalyse und Affinität in *Chem. Weekblad* **14**, 63, 1917.
46. Für die heterogene Katalyse im allgemeinen vergleiche man die rezente Zusammenfassung von E. F. ARMSTRONG und T. B. HILDITCH, *Chem. Ind.* **44**, 701, 1925 und auch die Arbeit von H. S. TAYLOR, *J. phys. Chem.* **28**, 897, 1925.
47. Es möge sogleich bemerkt werden, dass dieser Fall wahrscheinlich vorliegt bei der Dehydrierung von Ameisensäure von Bakterien der Koligruppe.
48. WIELAND, a.a.O.

49. Vergleiche hierzu den nächsten Teil.
50. Siehe hierfür: H. J. PRINS, Rec. trav. chim. 42, 473, 1923.
51. Einen analogen Fall findet man bei der Verseifung verschiedener Ester, die sowohl von Wasserstoffionen wie von Hydroxylionen beschleunigt wird. In saurem Medium ist jedoch nur die Wirkung der Wasserstoffionen, in alkalischem Medium nur die Wirkung der Hydroxylionen von praktischer Bedeutung.
52. Die Frage, ob man hierbei an das Protoplasma selbst oder an irgend ein den Wasserstoff übertragendes Enzym zu denken hat, ist in diesem Zusammenhang ganz bedeutungslos. Die wichtigen Versuche von Warburg, Meyerhof, Rubner u.a., aus welchen hervorgeht, dass Atmung und Gärung in hohem Grade an die Struktur der Zelle gebunden sind, berechtigen uns ganz allgemein von Protoplasma zu sprechen.
53. Bull. Soc. Chim. Biol. 6, 326, 1924.
54. Vergl. die neueren Arbeiten von W. RUHLAND, Ber. deutsch. bot. Ges. 40, 180, 1922 und G. GROHMANN, Centralbl. Bakt. Parasitenk. II Abt. 61, 256, 1924.
55. So werden in der Systematik der 'Society of American Bacteriologists' die wasserstoffoxydierenden Bakterien noch in ein Genus: *Hydrogenomonas* vereint. Vergl. BERGEY's Manual of Determinative Bacteriology, Baltimore 1923.
56. F. VISSER 'T HOOFT, Biochemische onderzoekingen over het geslacht *Acetobacter*. Diss. Delft 1925.
57. Bei der ersten Gruppe ist dies durch die Untersuchung von CLARA COHEN, Biochem. Z. 112, 139, 1920 und von C. NEUBERG und F. F. NORD, Biochem. Z. 96, 158, 1919 ebenfalls nachgewiesen. Für die sonstigen Gruppen ist die betreffende Literatur schon angeführt.
58. C. NEUBERG und F. WINDISCH, Biochem. Z. 166, 454, 1925. Nach den obigen Ausführungen braucht es wohl keiner Erläuterung, dass wir nicht mit der Auffassung der Autoren dieser interessanten Arbeit einverstanden sind, dass diese Dismutation des Azetaldehyds auch bei Anwesenheit anderer kräftiger Wasserstoffakzeptoren, wie Luftsauerstoff, also bei der normalen Essigsäurebildung, stattfinden wird. Wir kommen hierauf im 7. Teil zurück.
59. Dieser Vorgang liegt zweifellos vor bei den kräftig aeroben, fettbildenden Mikroorganismen. Vergl. hierzu: H. HAEHN und W. KINTTOF. Chem. Zell. Gew. 12, 157, 1925.
60. Bedingung für das Eintreten einer kräftigen Aldolkondensation, und damit auch für die Fettbildung, ist offenbar, dass der Azetaldehyd jedenfalls teilweise durch kräftige Lüftung vor einer Akzeptorwirkung geschützt wird. Dies steht in vollkommener Übereinstimmung mit den experimentellen Ergebnissen von Lindner, Haehn und Kinttof.
61. Diese 'karboligatische' Wirkung der Hefe gegenüber zugefügtem Azetaldehyd ist zuerst von C. NEUBERG und E. REINFURTH, Biochem. Z. 143, 553, 1923, nachgewiesen worden. Für die Dokumentierung obiger Anschauung vergleiche man: A. J. KLUYVER, H. J. L. DONKER und F. VISSER 'T HOOFT, Biochem. Z. 161, 361, 1925.

62. Nach unveröffentlichten Untersuchungen des einen von uns (D.)
63. N. L. SÖHNGEN, Centralbl. Bakt. Parasitenk. II Abt. 37, 595, 1913.
64. Vergl. z.B.: F. VISSER 'T HOOFT, Diss. a.a.O.
65. Man siehe z.B.: W. MANSFIELD CLARK, The Determination of Hydrogen Ions, Baltimore 1923, Chapter XVI.
66. Vergl. hierfür: S. KOSTYTSCHEW, Die Pflanzenatmung, Berlin 1924, S. 89.
67. O. MEYERHOF, Pflügers Archiv. f. d. ges. Physiol. 164, 353, 1916; ebenda, 165, 229, 1916; ebenda, 166, 240, 1917.
68. Ganz vollständig korrekt ist die gegebene Erklärung nicht, da vor allem die Vermehrungs- und weniger die Dissimilationsvorgänge bei diesen Bakterien durch die Zufügung organischer Substanzen verhindert werden. Dies bedeutet, dass eben die Kohlensäure, welche nur nach Aktivierung durch das Protoplasma als Akzeptor auftreten kann, von der fest an das Protoplasma gebundene organische Substanz verhindert wird, sich an das Protoplasma anzulagern. Vergleiche hierfür den nächsten Teil.
69. W. BAVENDAMM, Die farblosen und roten Schwefelbakterien. Jena 1924.
70. L. G. M. BAAS BECKING, Annals of Bot. 39, 613, 1925.
71. Diese Frage konnte hier nur kurz angedeutet werden. Die weitere wichtige Literatur auf diesem Gebiete – besonders die schönen Arbeiten Buders – müssen hier unberücksichtigt bleiben.
72. Oder Schwefel. Vergleiche Beijerincks: *Thiobacillus denitrificans*.
73. M. W. BEIJERINCK und D. C. J. MINKMAN, Centralbl. Bakt. Parasitenk. II Abt. 25, 30, 1910.
74. H. WIELAND in: Oppenheimers Handbuch der Biochemie, 2. Aufl. Jena 1923, Bd. 2, S. 265.
75. P. NAWIASKY, Archiv. f. Hyg. 66, 209, 1908.
76. Für die sehr verbreitete oxydative Dissimilation der Aminosäuren siehe man den nächsten Teil.
77. Jedoch gibt es auch hier Ausnahmen. So scheiden die Butylalkoholbakterien in der ersten Gärungsphase z.B. Buttersäure in das umgebende Medium ab. Nachdem sich diese Säure hierin angehäuft hat wird sie in der zweiten Gärungsphase wieder in den Stoffwechsel bezogen und in Butylalkohol übergeführt. Vergleiche die im 2. Teil bei der genannten Gruppe angeführte Literatur.
78. Dies hindert nicht, dass die Dissimilationsprozesse gegenüber den Assimilationsprozessen sowohl in materieller wie in energetischer Hinsicht meistens bedeutend vorherrschen, was vor allem auf Konzentrationsverhältnisse der in der Zelle anwesenden Substanzen zurückzuführen ist.
79. Vergleiche hierzu: T. THUNBERG, Svensk. Kemisk Tidskr. 145, 1923. Zitiert nach Wochenschr. f. Brauerei 41, 33, 1924. G. KLEIN und O. WERNER, Biochem. Z. 168, 361, 1926.
80. H. HAEHN und W. KINTTOF, Chem. Zell. Gew. 12, 115, 1925.
81. F. KNOOP und HUBT. OESTERLIN, Z. physiol. Chem. 148, 294, 1925.
82. Absichtlich ist hier der in den letzten Jahren von O. Meyerhof verkündigte Antagonismus zwischen Atmung und Gärung ausser Betracht gelassen. Dass die Muskelzelle nach Wiederherstellung der zeitweise unterbrochenen Aerobie

die angehäuften Milchsäure teilweise in Glykogen rückverwandelt, ist nur ein sehr auf der Hand liegender Spezialfall der Bildung eines typischen Assimilationsproduktes. Auch die Gärung ist zur Bildung derartiger Assimilationsprodukte durchaus befähigt und ein prinzipieller Gegensatz zwischen Atmung und Gärung ist hierin nicht zu sehen. Wir hoffen auf diese Frage später zurückzukommen.

83. a.a.O.

84. J. NEEDHAM and D. NEEDHAM, Proc. Royal Soc. Ser. B. 98, 259, 1295.

QUELQUES REMARQUES SUR LA CLASSIFICATION DES LEVURES

CE n'est que pour vous donner quelque'idée du travail du 'Centraalbureau voor Schimmelcultures', institution subventionnée par la Section de Botanique de notre union, que j'ose attirer votre attention sur quelques remarques inévitablement superficielles concernant la classification des levures. L'apparition récente d'une publication assez volumineuse de la plume de Mme Stelling-Dekker [1931] sur les levures sporogènes semble justifier un bref aperçu de ce qui a été fait dans le secteur des levures du bureau pendant les dernières années.

Pour ceux qui ne connaissent pas le catalogue du 'Centraalbureau', il faut observer que la collection du bureau, grâce à une coopération internationale remarquable, comprend un très grand nombre de cultures pures de levures. C'est bien ce fait qui nous a décidé entreprise très audacieuse d'entamer une révision critique de la classification des levures. Car il sera superflu d'insister sur le fait que la classification de ce groupe de microorganismes, qui par son importance pratique a attiré l'attention de tant de personnes d'une éducation scientifique limitée, se trouve encore dans un état plus ou moins chaotique.

Tout d'abord, il me semble indiqué de m'arrêter un instant à la question 'qu'est-ce qu'une levure'? Le mot vulgaire 'levure' ne laisse aucun doute sur son origine: depuis un temps immémorial on a donné le nom 'levure' au principe qui faisait lever les substances exposées à une fermentation alcoolique. C'est au génie de Pasteur que nous devons la connaissance de la nature biologique de ce principe et les recherches ultérieures ont bientôt montré la pluralité des microbes qui sont responsables du processus de la fermentation alcoolique. En outre une étude plus approfondie prouvait bientôt l'existence de champignons étroitement reliés à la levure de la vinification au point de vue morphologique et qui pourtant étaient dépourvus de la propriété de faire fermenter les sucres. C'est ainsi qu'on accepte aujourd'hui l'existence d'un groupe étendu de champignons auxquels le nom vulgaire 'levure' est applicable.

Conformément à cet exposé M. Guilliermond, autorité éminente sur ce groupe de microbes, a donné la définition suivante: 'On entend par levure, au sens botanique, tout champignon unicellulaire quelles que soient ses propriétés biochimiques, de forme ovale ou sphérique, qui se multiplie par bourgeonnement'.

Toutefois, il me faut observer que cette définition ne peut nous satisfaire complètement, car nous devons à M. Lindner, de Berlin, et à feu mon prédécesseur Beijerinck la découverte de plusieurs espèces de champignons qui ont tous les caractères des levures mentionnées jusqu'ici, avec la seule exception qu'ils ne se multiplient pas par bourgeonnement, mais par cloisonnement transversal.

A première vue on pourrait croire qu'il suffirait de modifier simplement la définition donnée par Guilliermond en ajoutant les mots: 'ou par cloisonnement transversal' pour l'adapter à la découverte des Schizosaccharomycètes. Mais en y réfléchissant, c'est clair que ce n'est pas ainsi, car l'addition des mots 'ou par cloisonnement transversal' comporterait que la grande majorité des bactéries satisferait à la définition de Guilliermond.

J'espère vous démontrer tout à l'heure que c'est justement cette difficulté de bien délimiter le groupe de levures qui est responsable de beaucoup de méprises.

D'abord, il me semble désirable d'essayer par une autre voie d'arriver à une définition scientifique de la notion 'levure'. La seule procédure praticable me paraît, de partir des propriétés d'une espèce de levure incontestable et de répondre alors à la question, quels organismes montrent des affinités suffisamment étroites pour les incorporer à l'espèce initiale dans le même groupe naturel.

C'est tout indiqué de choisir comme espèce initiale l'organisme qui provoque la fermentation spontanée du jus de raisins. Des centaines de recherches nous ont appris que cette expérience mène presque toujours au développement prépondérant d'une espèce de levure connue depuis longtemps sous le nom scientifique de '*Saccharomyces ellipsoideus*'.

Que savons nous maintenant des propriétés de cette levure? D'abord on peut observer que c'est un organisme qui se conforme bien à la définition donnée par Guilliermond. Mais il est évident que cette formule ne suffit pas pour établir la place qu'il faut lui assigner dans la classification des champignons. Il est donc d'une extrême importance que nous devons à Reess la découverte de l'existence d'un vrai cycle

évolutif de la levure de vin. C'est Reess qui a observé pour la première fois que cette levure ne se multiplie pas seulement par bourgeonnement, mais que dans certaines conditions la levure de vin donne lieu à la formation d'un sporange, dont les spores à leur tour sont capables de se transformer de nouveau en cellules levures typiques.

Les belles recherches morphologiques et cytologiques de Guilliermond montrent indubitablement que ces sporanges sont parfaitement les homologues de l'asque des *Ascomycetes*, en d'autres termes, que la levure de vin appartient à cette classe de champignons.

C'est ainsi que la question de la délimitation du groupe des levures s'est réduite à cette question: quels champignons appartenant à la classe des *Ascomycetes* sont suffisamment étroitement reliés à la levure de vin pour les rassembler dans un seul groupe naturel, celui des levures?

Ce sont encore les études admirables de Guilliermond qui nous ont montré le chemin pour répondre à cette dernière question. Il résulte de ces recherches que plusieurs espèces du genre *Endomyces* ne diffèrent de certaines espèces de levures typiques que par le fait qu'elles montrent aussi par la forme levure une forme mycélienne typique, ou plutôt que les formes levures ne sont que des conidies qui se multiplient par bourgeonnement. A leur tour ces espèces d'*Endomyces* montrent une relation étroite avec le champignon décrit par Mlle Stoppel sous le nom d'*Eremascus fertilis* dont la multiplication végétative cependant s'est restreinte à la forme mycélienne.

Mais il y a plus. On connaît aussi d'autres espèces d'*Endomyces* qui sont également reliées à l'*Eremascus fertilis* mais qui se distinguent des espèces mentionnées d'abord par le fait qu'on n'y rencontre jamais la formation de conidies. Au contraire ces dernières espèces sont caractérisées par le fait que leur mycélium se divise souvent en particules, en d'autres termes, il y a formation d'oidies. Il est évident que ce sont ces espèces dont on peut dériver en accentuant cette propriété ce qui mène à la réduction totale de la forme mycélienne, les espèces de levure, dont j'ai parlé plus haut, à savoir celles qui se multiplient par cloisonnement transversal.

Ce dernier résultat nous permet de définir le groupe naturel des levures comme un sous-groupe d'*Ascomycètes*, caractérisé d'un côté par la prépondérance plus ou moins absolue de l'existence unicellulaire et de l'autre par sa relation évidente avec l'*Eremascus fertilis*.

Il faut ajouter qu'on connaît d'ailleurs de nombreuses espèces qui dans leurs propriétés montrent une concordance frappante avec les levures ainsi définies, mais qui se séparent jusqu'à présent par le simple fait qu'elles sont incapables de produire des ascospores. Il est évident qu'il convient de ranger ces dernières levures parmi les *fungi imperfecti* de situation douteuse dans le système naturel. Ici je ne m'occuperai plus de ces levures imparfaites.

Vous vous demanderez peut-être pourquoi j'insiste si fort à préciser la notion 'levure'. Je réponds à cette question que l'expérience nous montre que ces idées – quoiqu'elles datent déjà de plus de vingt ans – ne sont pas généralement répandues même parmi les travailleurs dans ce domaine de la mycologie. Aussi n'est-il pas rare qu'on décrive comme étant de nouvelles espèces de levures des microbes qui en réalité sont très éloignés d'elles. Dans les dernières années le Centraalbureau a eu deux fois l'occasion de fixer l'attention sur de telles erreurs. D'abord il s'agissait d'un organisme décrit par le Docteur Benedek, de Leipzig, sous le nom de *Schizosaccharomyces hominis*. La découverte de cette espèce semblait intéressante entre autre parce que ce serait le premier exemple d'un Schizosaccharomycète habitant les régions tempérées. Toutefois une étude faite par Mlle Dorrepaal [1930] au laboratoire du Centraalbureau apprit bientôt que l'organisme de Benedek avait bien une ressemblance morphologique superficielle avec les Schizosaccharomycètes, mais qu'il n'y avait aucun doute que cet organisme n'appartient point aux Ascomycètes. Au contraire, il était évident qu'en réalité le *Schizosaccharomyces hominis* était une bactérie banale voisine du *Bacillus megatherium* de Bary.

Le second cas, encore plus récent, est parfaitement analogue. Seulement ici il ne traitait pas exclusivement de la création erronée d'une nouvelle espèce, mais même d'un nouveau genre de levures. Ici je fais allusion à la création du genre *Schizotorulopsis* par Ciferri, savant d'ailleurs bien connu par d'autres études approfondies sur les levures asporogènes. La recherche faite par Mlle Verkaik au laboratoire du Centraalbureau sur la seule espèce du nouveau genre: *Schizotorulopsis Alfonsocai* Ciferri établit aussi avec certitude que cet organisme n'était qu'une espèce de bactérie, remarquable par ses grandes dimensions sur certains terrains de culture. Ici la preuve de la nature bactérienne de l'organisme est spécialement victorieuse, parce que Mlle Verkaik [1931] réussit à démontrer que l'organisme est pourvu de motilité, si on le

cultive dans un milieu approprié. Conformément à cette observation la présence de fouets pouvait être prouvée.

J'ai insisté sur ces deux exemples, parce qu'ils montrent combien il est important d'avoir des notions claires sur la délimitation du groupe des levures, car il faut noter que le *Schizosaccharomyces hominis* autant que le *Schizotorulopsis alfonsecai* se conforment très bien à la définition courante donnée par Guilliermond, du moins quand on y apporte l'amendement inévitable sur la multiplication par cloisonnement transversal.

La tâche que Mme Stelling s'était posée, était donc un examen critique de toutes les cultures de levures sporogènes présentes dans la collection du Centraalbureau. Le nombre des espèces représentées, répandues sur 19 genres, se montait à 159 ou 73% du nombre des espèces, dont la description pouvait être trouvée après une enquête minutieuse de la littérature.

Le but des recherches de Mme Stelling était double: d'abord il fallait examiner si les propriétés des cultures se conformaient aux descriptions originales données par les auteurs. Mais en vue de la témérité avec laquelle un grand nombre de ces auteurs ont proposé de nouvelles espèces pour les levures isolées par eux il était aussi indispensable de se rendre compte si l'existence d'une telle espèce était suffisamment justifiée.

Le tableau peut donner quelque idée des grandes lignes de la classification que Mme Stelling a cru bon d'accepter pour le moment. Vous y retrouverez les pensées qui ont conduit M. Guilliermond dans ses considérations sur la phylogénie des levures. Sans me perdre dans les détails un bref exposé de la planche me semble être utile.

D'abord l'ensemble des levures sporogènes est réunie en une seule famille, celle des *Endomycetaceae*. Une séparation nette entre celle-ci et une seconde, à laquelle les levures dans un sens plus limité appartenaient: la famille des *Saccharomycetaceae*, nous a paru impraticable.

Néanmoins, il convient de distinguer dans la famille des *Endomycetaceae* quatre sous-familles. D'abord celle des *Eremascoideae* avec le seul genre *Eremascus*. Cette sous-famille est caractérisée – comme nous l'avons déjà observé – par l'absence d'autres formes végétatives que la forme mycélienne. Dans l'*Eremascus* donc il n'y a pas de conidies-levures et pas d'oidies. La seconde sous-famille est celle des *Endomy-*

coideae. Là, on trouve deux genres qui se distinguent par le fait que dans le premier: *Endomyces* on trouve à côté de la forme mycélienne la forme oïdie, tandis que dans le genre *Schizosaccharomyces* ce n'est que la forme oïdie qui subsiste.

La troisième sous-famille nous montre une situation analogue. Ici on trouve encore un genre dans lequel la forme mycélienne subsiste: c'est le nouveau genre *Endomycopsis*. Mme Stelling s'est décidée de réunir à ce genre toutes les espèces d'*Endomyces* qui en contraste avec l'espèce typique de ce genre, à savoir *Endomyces decipiens*. Ce sont là les espèces qui sont indiquées par Guilliermond comme étant les ancêtres des vraies levures bourgeonnantes.

Quant à ce groupe, qui comprend la majorité des espèces de levures décrites jusqu'à ce jour, il convient de distinguer encore deux sous-groupes. Tandis que pour la plupart des levures chaque partie de la membrane de la levure peut figurer comme point de départ pour la formation des bourgeons, quelques genres sont caractérisés par le fait que le bourgeonnement des cellules ovales s'accomplit exclusivement aux pôles. Cette particularité donne à ces levures un aspect typique auquel il faut aussi attribuer quelque valeur au point de vue de la classification. J'abuserais trop de votre indulgence en faisant la revue de tous les genres des tribus des *Saccharomyceteae* et des *Nadsonieae*.

Je me bornerai donc à faire encore quelques remarques générales sur les caractères qui se prêtent à la délimitation des genres et des espèces.

Vous aurez observé que pour les grandes lignes de la classification les propriétés morphologiques externes: forme mycélienne, forme oïdie, forme levure et type de bourgeonnement, nous ont suffi. Cependant il va sans dire que tous les genres ainsi délimités ont un caractère commun: celui de produire des ascospores. Aussi est-il facile à comprendre que les particularités du mode de formation des asques auront aussi une grande importance au point de vue de la classification.

Pour ceci il faut distinguer entre la formation d'asques par parthénogénèse et entre la formation d'asque précédée d'une copulation de deux cellules-levures. En outre pour les genres *Torulaspora* et *Schwanniomyces* il est typique que les cellules qui se transforment en asques essaient de copuler au moyen d'un ou plusieurs tubes sans y jamais parvenir. Dans le cas d'une copulation précédente il convient de différencier encore entre la copulation isogamique et hétérogamique.

Endomycetaceae

Eremascoideae

Eremascus

Oyd.



Endomycoidae

Endomyces

Oyd.
Firm.



Schizosaccharomyces

Firm.



Saccharomycetaceae

Endomycopseae

Endomycopsis

Oyd.



Saccharomyceteae

Saccharomyces

Saccharomyces s.s.



Nadsonieae

Saccharomycodes

Firm.



Hanseniaspora

Firm.



Nadsonia

Oyd.
Firm.



Nematosporoideae

Monosporella



Nematospora

Oyd.
Firm.



Coccidiascus

Oyd.



Pichia

Pichia s.s.

Oyd.



Zygophilia

Oyd.



Hansenula

Oyd.
Firm.



Debaryomyces

Oyd.



Schwanniomycetes

Firm.



Un cas spécial de la copulation hétérogamique est encore rencontré dans les levures du genre *Nadsonia*. Ici on trouve d'abord copulation entre une cellule-mère et une cellule-fille. Après l'accomplissement de la fusion l'oeuf forme au pôle opposé au gamète mâle un bourgeon dans lequel émigre son contenu et se transforme en un asque à un seul ascospore.

Enfin la forme et l'aspect des ascospores — présence d'un globule oléagineux, à paroi lisse ou verruqueuse — présentent aussi des caractères utiles pour la différenciation des genres.

Toutefois tous ces caractères de nature morphologique ne suffisent pas dans tous les cas pour la délimitation des genres. On connaît depuis longtemps des espèces qui ne montrent pas de différences essentielles au point de vue morphologique avec la levure de vin et d'autres espèces voisines, mais qui pourtant s'en distinguent nettement par leur conduite dans les milieux de culture liquides. Tandis que la plupart des espèces de levures cultivées dans une solution sucrée sont caractérisées par un développement diffus, qui se termine par la formation d'un dépôt, les espèces mentionnées plus haut végètent sur une telle solution en formant un voile mycodermique.

Il est évident que cette divergence est le symptôme d'une différence marquée dans le métabolisme des deux groupes. Apparemment les levures à formation de voile ont une tendance beaucoup plus accentuée à entraîner l'oxygène libre dans leur métabolisme.

Si l'on considère que l'étude des diverses espèces de levure est étroitement liée à celle des transformations biochimiques provoquées par ces organismes, il n'y a pas lieu de s'étonner du fait que les caractères physiologiques ont toujours eu une grande importance dans la classification des levures.

De même, il me paraît aussi qu'une telle procédure est entièrement justifiable. Puisque le métabolisme n'est qu'une réaction chimique du protoplasme vivant sur les substances nutritives présentes dans le milieu de culture, il convient de considérer les différences en métabolisme comme désignant des différences en propriétés de la partie la plus importante de la cellule vivante : le protoplasme.

Il est donc clair qu'on ne peut pas négliger dans la classification la grande différence qui existe entre les levures d'une dissimilation fermentative et celles d'une dissimilation préférablement oxydative qui se manifeste bien souvent dans la formation des voiles.

C'est ainsi que Mme Stelling, en accord d'ailleurs avec les auteurs précédents, a assuré la délimitation des genres *Pichia* et *Hansenula* avec les autres genres de la tribu des *Saccharomycetaceae*.

Enfin je dois faire la remarque que Mme Stelling s'est décidée de réunir en une quatrième sous-famille, à savoir celle des *Nematospori-deae*, les trois genres, souvent considérés comme des levures douteuses: *Monosporella*, *Nematospora* et *Coccidiascus*. Jusqu'à ce jour aucune espèce de *Monosporella* et de *Coccidiascus* n'a jamais été cultivée. Ces genres étaient donc inaccessibles à un nouvel examen. Quant à *Nematospora* les recherches récentes de Guilliermond donnent la preuve définitive de la nature ascomycète des espèces de ce genre. En outre, les descriptions de la formation d'asque et de la forme aiguille ou fuseau des ascospores dans *Monosporella* et *Coccidiascus* semblent bien justifier la réunion de ces deux genres avec *Nematospora* en un seul sous-groupe des *Endomycetaceae*.

Jusqu'ici je ne vous ai parlé que de la délimitation des genres. Ici la contribution de Mme Stelling se borne principalement à avoir donné des diagnoses plus achevées des genres déjà créés par les travailleurs antérieurs. Toutefois le travail du Centraalbureau s'est dirigé principalement vers l'examen critique de la validité des nombreuses espèces qui ont été décrites jusqu'ici.

Pour se faire une idée des difficultés qu'il fallait surmonter à ce sujet, il faut savoir que la plupart des mycologistes qui isolaient une levure dans des conditions un peu anormales ont cru avoir le droit de décrire cette levure comme étant une nouvelle espèce. Bien souvent tout effort a manqué pour comparer la culture isolée avec des espèces déjà existantes.

Il faut avouer que la description insuffisante et presque toujours incidente de la plupart des espèces rendait une telle comparaison extrêmement difficile sinon impossible. La situation était devenue peu à peu si chaotique que même les auteurs les plus compétents étaient incapables de donner un aperçu des différentes espèces réunies en un genre quelconque.

Il fallait donc une recherche expérimentale comparative des diverses espèces en faisant usage de méthodes plus ou moins standardisées pour éliminer la confusion existante.

La circonstance favorable que 72% des espèces décrites – et pour plusieurs des genres ce nombre était encore beaucoup plus élevé –

étaient représentées dans la collection du Centraalbureau, a permis à Mme Stelling d'entamer cette recherche.

Ce travail, dans lequel elle a été assistée par les demoiselles Dorrepaal et Verkaik n'a pas été en vain, ce qui résulte du fait que Mme Stelling donne dans sa monographie des arguments acceptables pour réduire le nombre des genres de 18 à 15 et le nombre des espèces étudiées de 159 à 85. En outre, elle a signalé dans sa monographie plusieurs exemples qui démontrent la nécessité de transférer une espèce d'un genre dans un autre.

Il en résulte que Mme Stelling peut donner pour chaque genre une clef dichotomique qui permet la détermination des diverses espèces du genre. Dès maintenant il est possible de donner une réponse plus exacte à la question s'il y a lieu d'identifier une levure nouvellement isolée avec une des espèces étudiées. Dans le cas opposé, on peut attendre que les diagnoses données par Mme Stelling faciliteront une délimitation exacte des nouvelles espèces. On peut même espérer que le travail exécuté stimulera la découverte de nouvelles espèces aux caractères intéressants; dans l'état actuel tout mycologiste hésite à ajouter une nouvelle espèce au grand nombre, que personne ne savait distinguer avec certitude. D'autre part – et voici ce qui est encore plus important – l'ordre atteint aura un effet préventif contre la création de nouvelles espèces qui en réalité n'ont aucun droit d'existence.

Qu'il me soit permis de faire encore quelques remarques sur les caractères acceptés par Mme Stelling pour la délimitation des espèces. Il n'est pas étonnant qu'il faille donner encore plus d'attention aux propriétés biochimiques, car il ne faut jamais oublier que le rôle des diverses levures dans la nature, ainsi que leur application industrielle, est déterminé en première ligne par le métabolisme de ces organismes.

Il va sans dire qu'à cet égard le type de dissimilation, soit oxydative, soit fermentative, est d'une importance primaire. Mais ce problème une fois résolu, il faut tenir compte du fait que les diverses levures montrent de grandes variations dans leur pouvoir d'attaquer les divers sucres.

Le mycologiste qui voudrait classer une levure aura donc à répondre à la question générale: l'organisme possède-t-il un pouvoir fermentatif et dans l'affirmative quels des sucres sont fermentescibles.

Voilà une question très facilement posée, mais on se trouve bien embarrassé d'y répondre.

Pourtant ce point est très important et il faut avouer que la non-chalance avec laquelle cette question a été traitée par la grande majorité des savants est responsable en partie de la confusion existante.

A ce point de vue, il est désirable d'examiner les méthodes en usage pour la détermination de la fermentescibilité d'un sucre.

Toutes ces méthodes sont fondées sur le même principe: on ensemente une solution de sucre avec la levure et constate s'il y a évolution de l'acide carbonique.

Toutefois en réfléchissant un peu sur ce critérium son insuffisance saute bientôt aux yeux. Car il est évident que non seulement la dissimilation fermentative, mais aussi la dissimilation oxydative (la respiration) mène à la production de l'acide carbonique. Il n'y a qu'une seule différence: c'est que l'effet énergétique de la combustion intégrale d'un sucre surpasse plusieurs fois celui de la fermentation de ce sucre. Ainsi la quantité du sucre transformé dans le cas de fermentation est toujours beaucoup plus grande que dans le cas de respiration. Il en est de même pour la quantité de l'acide carbonique produite dans les deux cas. Dans les levures respirantes cette quantité est toujours si minime que l'acide carbonique se dissout entièrement dans le milieu aqueux et il n'y a pas d'évolution visible de gaz. Dans le cas, au contraire, où la levure fait fermenter le sucre, la vitesse de la production de l'acide carbonique est généralement si grande que l'acide carbonique échappe du milieu à l'état gazeux.

Néanmoins faut-il conclure de ces considérations, que le résultat d'une telle expérience dépendra d'un côté de la vitesse avec laquelle l'acide carbonique est produit par la levure, et de l'autre de la vitesse avec laquelle l'acide carbonique peut échapper du milieu par simple diffusion. En vue du fait qu'on se sert d'appareils très divers pour l'examen de la fermentescibilité des sucres, il n'est pas étonnant qu'on rencontre bien souvent des résultats contradictoires dans la littérature.

La situation est encore aggravée par la circonstance que le développement récent de la physiologie nous montre que le pouvoir fermentatif d'une cellule est loin d'être un caractère absolu. Au contraire on sait qu'aussi des organismes d'une dissimilation oxydative prononcée, comme plusieurs des moisissures les plus ordinaires, sont capables de provoquer une fermentation alcoolique sous des conditions spéciales. Parmi ces conditions, l'absence de l'oxygène libre est probablement la plus importante.

A première vue, il paraît bien facile de tenir compte de cette dernière circonstance. Mais ici une autre difficulté se présente. L'expérience nous a appris que les levures sont incapables de se développer sous des conditions strictement anaérobies. Alors si onensemence un milieu anaérobie il ne se produira presque pas de développement de la levure et la quantité d'acide carbonique produite sera très limitée à cause du petit nombre des cellules qui provoquent la fermentation. Bien souvent cette quantité ne dépassera pas celle qui peut être dissoute dans le milieu et on conclura à tort une absence de pouvoir fermentatif. Si au contraire onensemence la levure dans un milieu auquel l'oxygène libre a accès, la levure peut se développer, mais dans ces conditions la respiration peut supprimer toute fermentation. Pourtant c'est bien possible que la même levure démontrera nettement son pouvoir fermentatif, si l'on rassemble une grande quantité de cellules dans un volume relativement petit d'un milieu sucré.

Un exposé de l'application des principes énoncée à l'examen critique des méthodes usuelles demanderait trop de votre patience; pour cela il faut se référer au livre de Mme Stelling.

Toutefois les considérations données suffiront pour démontrer qu'avec des levures qui ont une dissimilation fermentative peu prononcée on ne peut pas attribuer beaucoup de valeur au résultat des expériences de fermentation.

Il y a heureusement un grand nombre d'espèces qui ont un pouvoir fermentatif tellement accentué que l'oxygène à faible concentration ne peut point supprimer la fermentation.

Dans ce cas-ci il est d'une extrême importance d'observer l'action d'une levure vis-à-vis des différents sucres. Le résultat d'une telle recherche constitue alors un caractère spécifique d'une grande importance.

L'ensemble des résultats obtenus par Mme Stelling présente quelque intérêt, car il en résulte que la distribution du pouvoir fermentatif des diverses levures vis-à-vis des différents sucres n'est pas tout à fait capricieuse, comme la plupart des auteurs à ce sujet nous laissent croire. Au contraire quelques régularités à cet égard sur lesquelles j'ai déjà fixé l'attention en 1914 ont été bien confirmées par les recherches très étendues de Mme Stelling.

D'abord une levure ne fait jamais fermenter un sucre si elle ne fait pas fermenter la glucose. Deuxièmement pour toute levure qui fait

fermenter la glucose, la fructose et la mannose sont aussi fermentescibles. La troisième règle est que jamais une levure n'est capable de faire fermenter toutes les deux, la maltose et la lactose.

Aucun des nombreux exemples rapportés dans la littérature qui sont en contradiction avec ces règles n'a résisté à une inspection scrupuleuse.

En revenant à la question de la classification des espèces, il faut encore observer qu'aussi la manière dont les différentes levures se comportent vis-à-vis des milieux nutritifs synthétiques a fourni des données importantes.

Quant aux résultats concrets obtenus dans la révision des espèces je ne veux que citer quelques-uns à titre d'exemple.

En 1925 M. Redaelli a décrit sous le nom '*Saccharomyces cavernicola*' une nouvelle espèce isolée par lui des poumons d'une personne atteinte de la tuberculose. Une inspection minutieuse de la culture authentique nous a appris cependant que cet organisme était parfaitement identique avec le *Saccharomyces fragilis* de Jörgensen, une levure très répandue et qu'on presque toujours peut isoler du petit-lait.

Récemment M. Nishiwaki a décrit une nouvelle levure pour laquelle il a créé le nouveau genre *Zygosaccharomycodes*. La justesse d'une telle proposition a été déjà contestée par M. Guilliermond qui a assigné à cette levure une place dans le genre *Zygosaccharomyces*. En bonne harmonie avec cette opinion nos recherches ont montré l'identité du *Zygosaccharomycodes japonicus* avec la *Zygosaccharomyces mandshuricus*, levure décrite par Saito, il y a déjà quinze ans.

On peut espérer que l'augmentation de la possibilité d'identifier une levure contribuera aussi à une extension de notre connaissance de la répartition des diverses espèces.

Jusqu'ici l'opinion a été très répandue qu'à l'exception de quelques espèces ubiquitaires, la répartition de la plupart des espèces est très limitée. Le grand nombre de cas dans lesquels les noms spécifiques sont dérivés de noms géographiques font témoignage de cette manière de voir.

Toutefois nous avons déjà rencontré plusieurs exemples qui s'opposent à cette idée. Une levure, isolée du lait par nous et provisoirement décrite sous le nom *Saccharomyces galactosus* s'est montrée nettement identique à une levure isolée par Naganishi, à Dairen en Manschourie, il y a 14 ans et décrite par ce savant comme *Saccharomyces dairensis*.

Un second exemple est fourni par une levure reçue il y a quelques mois par le Centraalbureau de l'Île de Célèbes, aux Indes Néerlandaises. Cette levure n'avait aucun caractère qui permit de la distinguer du *Saccharomyces mangini*, levure rapportée par la mission Chevalier de l'Afrique Occidentale.

Je vais conclure, j'ai déjà trop exigé de votre patience. Je serai bien heureux si mon exposé vous a convaincu que le Centraalbureau est une institution qui mérite bien l'appui de notre Union. Mais surtout je vous serai extrêmement reconnaissant si vous voulez bien après votre retour chez vous, animer vos collègues-mycologistes de donner leur coopération précieuse aux travaux du Centraalbureau.*

* *Ed note:* This paper was published from a manuscript used for oral delivery, and Professor Kluyver did not have an opportunity to smooth out certain passages before it appeared in print. He often expressed his regrets about this development.

The Editors have made some minor alterations in the original version which, they believe, are in accordance with the intentions of the author.

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PROSPECTS FOR A NATURAL SYSTEM OF CLASSIFICATION OF BACTERIA

(WITH C. B. VAN NIEL)

I. MOTIVES FOR A RENEWED DISCUSSION OF THE SUBJECT

At the present time only two systems of bacterial classification are in more general use. In Europe the system of Lehmann and Neumann [1926] is still prevalent, whilst in America many bacteriologists adhere to the system given in Bergey's Manual of Determinative Bacteriology [1934]. Yet it is certain that several investigators have felt that these systems are unsatisfactory both from a practical and from a taxonomic standpoint.

As a consequence several new or amended systems have been proposed which, however, have failed to draw the attention of the majority of bacteriologists. Amongst these there are a few contributions which well deserve a wider appreciation, because they are based upon what seems to the authors of the present essay a sound realization of the principles of scientific taxonomy. These studies often give evidence of a profound knowledge of the literature pertaining to the subject. Nevertheless, it is to be regretted that the most notable of the authors seem to be unaware of the existence of similarly directed efforts. Hence valuable suggestions offered by one author have not been considered by the kindred writers, although these might have materially aided in ensuring the success of the various attempts.

Under these circumstances it seems profitable to expound again the guiding principles of a rational taxonomy, to point out the deficiencies of the recently proposed systems, and finally to develop a classification which is largely built on the meritorious elements of many preceding studies and in which, on the other hand, fallacies inherent in many of these are avoided.

2. PRINCIPLES IN BACTERIAL CLASSIFICATION

If one examines the various systems of bacteria proposed up till now, it becomes evident that many of these systems are almost entirely the outcome of purely utilitarian motives. Very often such artificial systems are ultimately impractical because as a rule newly discovered facts necessitate profound modifications or even the construction of new systems at short intervals.

Naturally, the only truly scientific foundation of classification is to be found in an appreciation of the available facts from a phylogenetic point of view. Only in this way can the natural interrelationships of the various bacteria be properly understood. It has to be admitted at once that, inasmuch as the course of phylogeny will always remain unknown, the basis of a true phylogenetic system of classification will be very unstable indeed. On the other hand it cannot be denied that the studies in comparative morphology made by botanists and zoologists have made phylogeny a reality. Under these circumstances it seems appropriate to accept the phylogenetic principle also in bacteriological classification.

The question then arises in which characters phylogeny expresses itself. There is no doubt that in this respect morphology remains the first and most reliable guide. It is, however, a commonplace in systematic bacteriological literature to bewail the scantiness of suitable morphological data. This, in turn, is chiefly responsible for the unsatisfactory state of bacterial taxonomy.

This situation has, already a long time ago, induced several authors to apply characters of a physiological nature in addition. The first steps in this direction were made timidly, but gradually the defenders of the good right of a physiological basis for taxonomy have become more and more numerous.

It seems superfluous to dwell upon this evolution here. It may suffice to remark that nowadays the indispensability of physiological characters for the purpose of classification has been generally accepted, which is only natural because, after all, these physiological differences must be considered as expressions of variations in submicroscopical morphology.

In this connexion the predominant problem is only how the macro-morphological and the micromorphological (physiological) characters

should be rated. On the one hand we may refer to Orla-Jensen's [1909] classification which is an extreme example of the concept that physiological characters should determine the main lines of the system. In contrast herewith Prévot [1933] recently formulates one of his laws of bacteriological systematics as follows: 'les caractères physiologiques sont des caractères spécifiques', thus forbidding the use of physiological characters for the demarcation of units of higher systematic rank than the species. However, in another passage of his treatise Prévot obviously takes exception to his law by allowing for the creation of genera on a physiological basis in such cases where otherwise the morphological genera would become overburdened.

Personally we are of the opinion that Prévot rightly emphasizes the priority of morphological over physiological characters. Yet his restriction that the application of the latter should be confined to delimitation of species is of a quite arbitrary nature. In accepting the taxonomic value of physiological characters it cannot be understood why they could not also be applied for the demarcation of higher systematic units.

In support of this view we wish to observe that such a procedure is not at all limited to bacteriological classification. A typical example is offered by algologists who do not hesitate to support the separation of the class of *Heterocontae* from that of the *Isocontae* by such typical physiological features as the nature of the storage products, the proportion of the pigments present, and the chemical constituents of the cell wall [West and Fritsch, 1927].

There is, moreover, a second reason why it is only rational to use physiological characters not exclusively for differentiation of species but also for uniting them into higher groups. This reason is to be found in the fact, that the mere act of cultivating a bacterium, of thus gradually familiarizing oneself with the various aspects of the organism, leads to a more or less unconscious inclusion of the physiological characteristics in fixing its systematic position. In other words, no bacteriologist can be satisfied with a classification which combines in one genus organisms which behaved quite dissimilarly in his preliminary work. To cite just one example: the microscopical appearance of some of the aerobic sporeforming bacteria and that of certain types of butyric acid bacteria may show a striking resemblance, yet the technique in handling and the nutritional requirements of both groups are so obviously

different that it will be distasteful to the majority of bacteriologists to incorporate these organisms into one and the same genus.

One is thus led to the conclusion that among the physiological characteristics there are some which should be used in separating larger groups and the question is which ones may be deemed to be essential in this respect. Now it is clear that differences in nutritional requirements are conditioned by differences in the metabolic activities of the organisms. Thus the problem resolves itself in grading the metabolic properties according to their intrinsic value. A lack of insight in the fundamentals of metabolism has thus far been the great stumbling-block for a rational application of physiological characteristics in taxonomy and it also explains the horror with which many systematians have witnessed their ever increasing use.

In surveying the metabolism of the various bacteria one is struck by its great diversity which contrasts sharply with the relatively great uniformity characteristic for the metabolism of higher plants and animals. An analysis of this situation bears out that, whereas the anabolic processes are essentially similar in both cases the fundamental differences are to be found in katabolism. It is especially the many different ways in which bacteria succeed in meeting their energetic requirements which draw the attention. Besides the respiration process, as it is also found in higher organisms, we encounter numerous instances in which this process is substituted for by conversions in which free oxygen does not take any part. Moreover, all these processes are characterized as well by the diversity of their substrates as by the different ways in which these substrates are converted into the final products of katabolism.

The fundamental nature of the energy providing processes justifies the view that they should be rated first amongst the physiological characters suitable for classification, the more so since these properties are typically reflected in the cultural behaviour of the organisms.

If, in consequence of this, one is led to the creation of systematic groups on the basis of katabolic properties is not the principle of phylogeny as a taxonomic basis endangered? This needs not be the case provided this principle is duly subordinated to the requirements ensuing from a primarily morphological classification. For it does not seem excluded at all that in a single morphological group a physiological evolution is responsible for the differences in katabolism observed,

whereas parallel evolutionary processes may have taken place in various morphological units.

One might object to this hypothesis, since the differences in katabolism seem to be of such an absolute nature as to exclude any evolutionary idea. However, recent development in the study of bacterial metabolism seems well suited to modify this view. A closer analysis of the katabolic processes has revealed their fundamental unity and made it possible to connect the differences observed largely with differences of a quantitative nature in some special property of the living cell [Kluyver, 1931].

This quantitative gradation implies that also the physiological groups do not show any sharp lines of demarcation. Hence, the boundaries imposed by the intuition of the investigators will always bear a more or less arbitrary character. The situation seems comparable with that existing in colour distinction. Although every one will accept the validity of such clearly distinguishable colours as red, green, blue, yet a glance at the spectrum is convincing evidence that also in this case boundaries are lacking.

It is obvious that the resulting taxonomic difficulty will be felt most severely in the determination of the ultimate systematic unit, the species. The masterly way in which Benecke [1912], as early as 1912, has expressed himself on this matter justifies the quotation in extenso of the following passage.

‘Was sind nun die Arten, d.h. die niedrigsten von den eben aufgeführten systematischen Einheiten?

‘Die Antwort lautet: Das, was der Forscher, welcher die Art aufstellt, nach seinem “wissenschaftlichen Takt” darunter zusammenfasst. Anders kann die Frage offenbar darum nicht beantwortet werden, weil die Natur selbst keine Arten kennt, sondern nur Individuen mit ihrer Aszendenz und Deszendenz, also nur sog. “Linien”, und solche Linien fasst eben der Systematiker zu Linienbündeln zusammen, die er Arten nennt. Wie gross oder wie klein er sein Bündel schnüren will, das hängt von seiner wissenschaftlichen Auffassung ab, die von der eines anderen mehr oder minder abweichen kann. Freimachen von dieser subjektiven Umgrenzung der Arten würde sich der Systematiker dann, wenn er auf noch niedrigeren Einheiten als den Arten fassen wollte, eben jenen Linien. Er müsste dann alle Bakterien in Form von Einzelkulturen züchten, und zwar unter den denkbar verschiedensten Be-

dingungen, unter denen sie überhaupt zu leben vermögen, würde sich all ihre Formen und Eigenschaften in Abhängigkeit von diesen Bedingungen merken und sie in die Diagnose der betreffenden Linie aufnehmen. Alle die reinen Linien, die dann keine Unterschiede in der Diagnose aufweisen würden, müsste er zu systematischen Einheiten zusammenfassen, zu sog. elementaren Arten, und diese von willkürlicher Umgrenzung wenigstens einigermaßen freien Einheiten nach Gutdünken zu höheren Einheiten zusammenfassen. Tatsächlich ist das nicht möglich; das braucht nicht weiter begründet zu werden, denn jene eben skizzierte Arbeit würde kein Ende absehen lassen. So müssen denn für die praktischen Zwecke der Systematik, d.h. um eine Übersicht über die Formen zu ermöglichen, höhere systematische Einheiten gewählt werden.'

Hence it follows that in an attempt to subdivide the organisms belonging to one 'natural' group of bacteria into species one shall have to create as many species as there are organisms which differ in 'sufficiently fundamental' characters, regardless of the possible existence of intermediate types. It depends entirely upon the 'scientific tact' of the investigator which characters will be deemed worthy of the designation 'sufficiently fundamental'.*

It is here the place to give a short survey of the characters which may be taken into consideration in this respect.

a. Morphological characters

It is self-evident that the shape of the cells is of outstanding importance for determining the place of a bacterium in any phylogenetic system. The same holds, of course, for the mode of reproduction and the occurrence of special resistant stages such as endospores, gonidia etc. In addition to these characters the size and the structure of the cells may give valuable indications.

With regard to structure it is especially the presence of organs for locomotion, flagella, and the way in which they are attached to the cell which has long been recognized as affording insight in mutual relationships. In this connexion it must, however, be remarked that experience has taught that in cases of immotility of the cells we have to discriminate between 'incidental' and 'genuine' immotility. For, as has

* It goes without saying that similar difficulties will also be encountered in marking off the higher systematic units, though to a lesser extent.

been emphasized by Orla-Jensen, we sometimes meet with immotile species which unquestionably are closely related to motile ones, whereas, on the other hand, one is struck by the fact that in several large natural groups motility is completely lacking

Finally one diagnostic character of wide application, *viz.*, the Gram stain, may also be considered as dependent on structural properties of the cell and the many instances of a close correlation of the outcome of the Gram test with several other characters leave no doubt as to its significance also for taxonomic purposes.

b. Physiological characters

The behaviour of the various bacteria in relation to temperature and osmotic pressure of the environment has often been used in the characterization of species. Recent investigations tend to throw doubt upon the soundness of this procedure, because of the wide range of adaptation which is manifested by most bacteria.

As has already been set forth the physiological characters of prime importance are those which are connected with the metabolism of the organism. Although in this respect the significance of the katabolic properties of the cells has been rightly stressed, yet one should not lose sight of the fact that in some cases the occurrence of special products of anabolism can also be made use of.

A first division of katabolic activity has to be made according to the source of the energy required by the cell. Here we must distinguish between the organisms capable of utilizing radiant energy and those which are dependent upon the chemical energy supplied by one or more constituents of the medium. In the latter case, which is the more common with bacteria, a further subdivision imposes itself according to the role of the free oxygen in katabolism. Firstly we find bacteria in which this gas is as indispensable as it is for higher plants and animals. But in addition to this category there exist organisms which can satisfy their energy requirements also in the absence of oxygen. Finally there is a third group of bacteria, which thrive only in its absence, or nearly complete absence.

In each of these groups a further subdivision can be made both on the basis of the nature of the favoured katabolic substrates and on the basis of the mode of decomposition as evidenced by the nature of the final katabolic products. It may be added at once that this predilection

for a special group of substrates – carbohydrates, protein decomposition products, or organates – involves an adaptation to life in an environment of a special range of hydrogen ion concentration.

It must be pointed out, however, that a judicious selection of the criteria on which the above mentioned subdivision should be founded, is most essential. In this connexion it cannot be sufficiently stressed that one should not confuse the physiological significance of the ability to derive energy from the desmolytic transformation of a special group of substrates with the ability to hydrolyze compounds of a complex nature, like proteins, polysaccharides, fats etc. For it must be clear that there exists a far more fundamental difference between an organism which splits glucose into lactic acid and a second organism which produces out of the same substrate butyric and acetic acid, carbon dioxide and hydrogen, than between two lactic acid bacteria only one of which attacks maltose. In the latter case both organisms derive their energy from the same conversion of glucose into lactic acid, the hydrolysis of the maltose into glucose being only an introductory act devoid of any energetic significance. Differences in the hydrolytic capacity should never be applied for distinguishing systematic groups, they can merely be taken into account for the differentiation of species.

The neglect of this point of view is undoubtedly responsible for the dislike with which morphologically inclined taxonomists view the application of physiological characters for the delimitation of systematic units larger than species.

Finally some remarks should be made regarding a physiological character of rather wide application in present day taxonomy, *viz.*, pathogenicity. This appears to be a character of very doubtful value. For the case is not rare that a pathogenic organism is so closely related to a non-pathogenic one that the two are undistinguishable except with the aid of infection experiments. The creation of separate genera on the basis of such a character is objectionable, because this implies that even the generic nature of an organism cannot be decided upon independently of a knowledge of its previous history. The same difficulty holds, albeit to a lesser extent, for a differentiation of species on the basis of pathogenicity.*

* Cf. particularly the valuable remarks made by O. Rahn, Zentralbl. Bakt. Parasitenk. Abt. II. 78, 1, 1929; 79, 321, 1929; and Stapp, C., Schizomycetes (Spaltpilze oder Bakterien) in: Sorauer, Handb. d. Pflanzenkrankh. Bd. 2. 1928. S. 1-295.

In summarizing the above it is tempting to conclude that on the one hand phylogeny has led to the origin of various morphological groups, whilst, on the other hand, an evolution in metabolic properties has occurred which together are responsible for the almost unlimited diversity of bacterial species. Before attempting to apply these principles in the construction of a system, we will first critically examine the more recent contributions to the classification of bacteria.

3. CRITICAL EXAMINATION OF RECENT CONTRIBUTIONS TO THE CLASSIFICATION OF BACTERIA

The large number of papers in which ideas on bacterial taxonomy are advanced makes an endeavour to give a moderately complete survey altogether impossible. Therefore we shall confine ourselves to a consideration of the more strictly taxonomic publications.

The system of Lehmann and Neumann [1926], adapted to modern needs since its inception in 1896, may still lay claim to a serious consideration. The outstanding feature, and at the same time the weakness of the system, is undoubtedly its simplicity. Although for the separation of the families of the *Schizomycetales* morphological characters are used exclusively, it would seem that a far better use could be made of such characters to express natural relationships. In this case the *Desmobacteriaceae*, which have nothing in common with the other five families, would have been set aside as a group equivalent to the orders of *Schizomycetales* and *Actinomycetales*. The same holds for the family of the *Spirochaetaceae*. The remaining four families seem at first sight acceptable as units resulting from a gross subdivision. Yet it appears from a closer inspection of the accepted genera that their grouping violates the principle of natural relationship in many respects. Thus one finds in the large, physiologically very heterogeneous, genus *Bacterium* organisms with polar flagella which are much more closely related to the family *Spirillaceae* than to the greater part of the other *Bacterium* species. Furthermore, the unmistakable relationship between the species of the genus *Plocamobacterium* with those of the *Proactinomycetaceae* is fully neglected, even to the extent of incorporating them in different orders. As regards the family of the *Desmobacteriaceae* its extremely heterogeneous nature should be emphasized. Apart from the fact that Lehmann and Neumann apologetically incorporate

in this family several large groups of bacteria not possessing the diagnostic characters of the family (*Thiobacillus* species, purple sulphur bacteria, the coccoid iron bacteria described by Molisch, etc.) there is no doubt that the recognized genera: *Beggiatoa*, *Leptothrix*, *Crenothrix*, *Cladothrix* and *Thiothrix* show neither affinities to the remaining genera of the *Schizomycetales* nor to one another.

It is clearly evident that in proposing this family Lehmann and Neumann have more or less unconsciously used physiological characters. Obviously the main reason for uniting the organisms in question into one family is not so much their morphological similarity as the peculiarity of their metabolism.

The most outstanding contribution of the system of the German authors and the one which has found general recognition is undoubtedly the realization of the close affinities existing between the genuine *Actinomycetes* and the genera *Corynebacterium* and *Mycobacterium*.

A revolution in the principles of bacterial taxonomy was brought about in the years 1908 and 1909 by the appearance of Orla-Jensen's publications [1908, 1909]. For the first time the classification of the bacteria was based mainly on the physiological characters. The idea underlying the proposed system is a phylogenetic one. A genealogy is developed as a result of considerations concerning the succession of physiological types in the course of the evolution of life on earth.

Migula's influence is observable in the emphasis placed on the significance of the difference in flagellation. The mode of insertion of the locomotive organs is used for the separation of the bacteria into two orders: the *Cephalotrichinae* and the *Peritrichinae*. A place was assigned to the immotile bacteria in either one of these orders on the basis of their physiological relationship to motile species.

Orla-Jensen [1921] has returned to the subject in a paper in which he makes another eloquent plea for his main theses. With the exception of a few minor amendments the system as proposed in 1908 is maintained.

The system has the great merit that it is built upon a sound evaluation of what is fundamental in the various physiological characters as manifested by the value attached to the energetically important katabolic properties. Another meritorious feature of Orla-Jensen's system is the creation of several new genera for physiologically well defined groups, together with the introduction of a rational code of

nomenclature for all genera, fully independently of the principle of priority.

On the other hand, it is not surprising that the manifest neglect of the importance of morphological characters other than the flagellation has given rise to severe criticism. Without entering into a discussion of the numerous objections raised, it may suffice to give here a few examples in which in consequence of this the principles of natural relationship have been violated. The place of the immotile groups in the system is not satisfactory. Here physiological characters, like special nitrogen requirements, have been decisive in the position of the genera *Streptococcus*, *Caseobacterium*, and *Propionibacterium* amongst the *Peritrichinae*, in the position of *Mycomonas*, *Corynemonas* and *Actinomyces* amongst the *Cephalotrichinae*. The reason for this is that Orla-Jensen has adopted the view that one and the same physiological evolution has occurred only once in phylogeny. The possibility is not considered that such an evolution may have taken place independently in various morphologically different groups.

Morphologically the three first mentioned genera show such close affinities to the genera *Mycobacterium* and *Corynebacterium*, that the grouping of these five genera in the two different orders is unacceptable. In addition the designation of the two last mentioned genera as *Mycomonas* and *Corynemonas*, thus suggesting a relationship between the representatives of these groups and the cephalotrichous genera, has its foundation exclusively in the oxidative character of their katabolism. Here again we encounter the idea that in determining the systematic position of a group metabolism dominates over morphology.

Another instance of artificial grouping is offered by the creation of the genus *Carboxydomonas* for the permanently immotile *Bacillus oligo-carbophilus* discovered by Beijerinck and Van Delden. The systematic position given to this organism by Orla-Jensen – also expressed in its new generic name – is merely based on the idea, that there is a close resemblance between the metabolism of the organism in question and that of the representatives of the genera *Hydrogenomonas* and *Methanomonas*. Morphologically, however, the species of the latter genera are so typically related to the *Pseudomonas* species and so distinctly different from *B. oligocarbophilus* that Orla-Jensen's classification has to be rejected.

In spite of the criticisms given we want to emphasize that in our

opinion Orla-Jensen's contribution to systematics – the prominent place assigned to rightly evaluated physiological characters – marks a milestone in the development of bacterial taxonomy.

In their entirety Orla-Jensen's views have never been accepted. It is the merit of Buchanan to have advocated a system of classification in which a limited use has been made of Orla-Jensen's ideas. In subdividing the order of the *Eubacteriales* Buchanan recognizes one family exclusively on the basis of physiological characteristics. The autotrophic nitrifying bacteria which Orla-Jensen considered as representatives of the most primitive among micro-organisms were here united in the family *Nitrobacteriaceae*.

Buchanan's system was more or less the foundation of that which soon afterwards evolved out of the work of a committee appointed by the Society of American Bacteriologists, of which Buchanan originally was a member. For a description of the development of the work of this committee we may refer to the data presented in Buchanan's [1925] most valuable, exhaustive monograph on bacterial systematics. It may suffice to state that the results have ultimately been laid down in Bergey's [1923, 1925, 1930, 1934] well-known Manual of Determinative Bacteriology. In the following discussion we will therefore limit ourselves to a criticism of the system as developed in the last edition of this book. As for the earlier editions we will only remark that in these – especially in the first one – the tentative character of the outlined system was duly emphasized in the preface. Gradually the attitude of the editing committee has, however, changed and has grown more and more self-confident, probably owing to the commercial success of the book. This should be regretted because the publication of this cooperative effort has led to an abundance of sound criticism which might have been usefully incorporated in later editions. By ignoring this criticism the beneficial effect which might have resulted from the cooperative character of the work – the first formal cooperation in the history of bacterial taxonomy – has been more or less nullified.

The final outcome can best be described as a compromise between the most divergent ideas which have been expressed in the course of time. Anyone who has had the opportunity to peruse the book will have been struck by the fact that morphological, physiological, nomenclative, utilitarian, cultural and pathogenic properties have been used

in the building up of the system in the most arbitrary way. The result is a complete lack of homology in the various groups, as has already been emphasized by Prévot (*l.c.*). In addition to this serious shortcoming an utter disregard for the significance of mutual relationships between natural groups is apparent.

In support of these statements the following instances may be cited. The first one of the five families of the order *Eubacteriales*, *viz.*, the *Nitrobacteriaceae*, is a curious conglomerate of organisms the majority of which have nothing in common save the fact that the average – usually medically trained – bacteriologist is unfamiliar with them. Whereas Buchanan, in creating this family, clearly intended to separate the nitrifying bacteria with their remarkable autotrophic mode of life from those bacteria for which organic substances are a necessary prerequisite for their development, this principle was violated when the Committee included in this family organisms which ‘may use in their metabolism’ also ‘simple carbon containing compounds’. Apart from the question what one has to understand by the restriction ‘simple’ it will suffice to remark that nearly all bacteria will answer this requirement. Nor can such a ‘simple’ metabolism have been meant to be imperative since any bacteriologist acquainted with genera like *Acetobacter* and *Rhizobium* will cultivate representatives of the genera in media containing an abundance of complex organic compounds!

The next family, *Coccaceae*, shows a clear-cut example of lack of homology in its subdivision in genera. No argument whatever is advanced for the sudden use of the character of pigment formation in the demarcation of the genus *Rhodococcus*. This emphasis on the occurrence of a red pigment in representatives of this genus, although nothing is known about a possible metabolic significance of the pigment, is most astounding, since the occurrence of a yellow or even an orange pigment amongst members of the genus *Micrococcus* does not seem to offer any ground for separating these from the non-pigmented species.

Proceeding to the third family, *Spirillaceae*, we will only point out that here again we meet with an instance where the occurrence of a red pigment is not considered of sufficient importance to be used as a generic character, for *Spirillum rubrum* Esmarch is classified together with the colourless spirillae. Unfortunately in this case – and this in contrast to what has been remarked regarding the *Rhodococcus* species – there would have been every reason for separating *Sp. rubrum* from the

other species, because here the pigment fulfils a fundamental metabolic function, *Sp. rubrum* being a typical purple bacterium. A documentation of this insight is in this case quite superfluous since this opinion is fully shared by Bergey *et al.* as is proved by the fact that we meet with the same organism again in an entirely different order (!) as *Rhodospirillum rubrum* (Esmarch) Molisch.

The next family, *Bacteriaceae*, offers no end of examples of heterogeneity, arbitrariness, utilitarianism, and disregard for natural relationship. The subdivision of this family into its twelve tribes has obviously been dictated by the tendency to keep apart the bacteria of importance to the hygienist from all others. In the key to the tribes this is obtained by giving the attribute 'pathogenic for animals' to the representatives of the former group. This leads to the remarkable situation that in this group of 'pathogens' several utterly harmless organisms, like many *Aerobacter* and *Alcaligenes* species, are encountered. Apart from this lack in consistency, making the organisms in question fully indeterminate, no bacteriologist who isolates a bacterium from soil, water, dairy products etc. will ever be able to decide whether it belongs to the 'would-be pathogens' of Bergey *et al.*. Hence another group of organisms, like the *Escherichia* species, becomes in most cases also indeterminate. The attempt to reinforce the antithesis pathogenic-non-pathogenic by ascribing to the former group an optimum temperature of 37.5 °C, against one of 30 °C or less to the latter group, shows the same lack of consistency as regards its practical application. Numerous are the instances in which species with low temperature optima are found in the so-called pathogenic group, whereas the authors also do not hesitate to include bacteria with a temperature optimum of 37 °C or even higher (*Serratia* spec., *Lactobacillus* spec.) in the saprophytic group. Moreover in the species diagnosis several organisms of this group are reported to be pathogenic or having the intestinal canal as their normal habitat!

If one now proceeds to the subdivision of the large group of saprophytes or plant parasites one is struck by the miscellany of characters which are used in the demarcation of the various tribes. One semi-morphological property, the outcome of the Gram-stain, is used; the other characteristics are all physiological and of a very dubious nature, such as the ability to grow either well or poorly on ordinary media, the ability to digest cellulose or not, etc. Attention should be drawn

furthermore to the great importance attached to the character of pigment production. Whilst in genera like *Spirillum*, *Propionibacterium*, *Mycobacterium* the production of yellow, orange, brown and red pigments is not considered to be of sufficient importance for making generic distinctions, here a special tribe is created for uniting the pigmented forms which are then divided into four genera on the basis of the colour. The occurrence of yellow pigments which in the genus *Micrococcus* is deemed inessential is here raised to a character of generic rank (*Flavobacterium*). Not all bacteriologists will realize, moreover, that for the correct determination of the common fluorescents he must reject the designation of the pigment as yellow but characterize it as green or blue-green. If he succeeds in avoiding this pitfall he will still be lost in case he is dealing with one of the numerous representatives of the genus *Phytomonas* which also produce a fluorescent pigment. For it is impossible to arrive at the last mentioned genus without this time having designated the same colour as pale yellow!

The arbitrariness may have been sufficiently demonstrated by the foregoing examples. Yet it is worth-while to note two more instances which at the same time clearly show the lack of homology in the various systematic units. In the tribe *Erwineae* the two genera are distinguished by the mode of insertion of the flagella; on the contrary the genera *Flavobacterium*, *Chromobacterium* and *Achromobacter* contain organisms with polar as well as such with peritrichous flagella. The second instance is the arbitrary use of the character of the Gram-stain. It has been mentioned above that the main subdivision of the saprophytes is based on this property; on the other hand in the group of animal parasites we encounter Gram-positive and Gram-negative species within the same genus (cf. e.g. *Alcaligenes*, *Bacteroides*).

We shall not continue the detailed criticism of Bergey's classification of the *Eubacteriales* but only make a few remarks regarding the remaining orders, documenting our opinion that in these orders the same inconsistency, lack of homology etc. are to be found. The second order, *Actinomycetales*, owes its origin to the just evaluation by Lehmann and Neumann of the natural relationship between the *Actinomycetes* and the bacteria of the genera *Mycobacterium* and *Corynebacterium*. The common characteristics of these groups are morphological and comprise positive Gram-stain, the lack of ability to form true endospores and permanent immobility. These properties are indeed included in the

diagnosis of the order as outlined in Bergey's manual. It is therefore perplexing to find in the family *Mycobacteriaceae* Gram-negative as well as motile organisms. Any one isolating an organism answering the diagnostic requirements of the genera *Mycoplana*, *Cellvibrio* and *Actinobacillus* would not hesitate a moment to include his organism in the order of the *Eubacteriales*.

A similar example is furnished by the species *Leptothrix hyalina* which, although it is stated not to form a sheath, is incorporated in the order of the *Chlamydobacteriales* regardless of the fact that in the key to the orders the occurrence of a sheath is decisive for including the organism in the order in question.

As for the next order, *Thiobacteriales*, we will only state that here, in contradistinction to what holds for the foregoing orders, morphological characters are fully left out of consideration in delimiting the order. The essential metabolic function of the sulfur or (and) the photosynthetic activity with the aid of the bacteriopurpurin pigment complex serve to characterize the order. Considered from this point of view it is rather surprising that the genus *Thiobacillus* is not included in this group.

With these comments on one of the most widely used systems the deplorable present state of bacterial classification is sufficiently illustrated.

During the course of the development of the American system a number of important studies on bacterial taxonomy appeared. One of the most outstanding contributions to a solution of the problem is undoubtedly the paper by Pringsheim [1923]. This publication contains several very sound general considerations on the principles of taxonomy with corresponding critical remarks on the previously proposed systems. We will confine ourselves to a brief review of the scheme as advocated by the author. Then we meet with a first subdivision of the bacteria into five orders, each one of which may be called homogeneous and homologous.

The principal feature of Pringsheim's subdivision of the first order, *Eubacteriales*, is unquestionably the fact that the usual, somewhat primitive, classification on the basis of spheres, rods and spirals is not strictly maintained inasmuch as the *Spirillaceae* also include the rod-shaped bacteria with polar flagella, *i.e.* the genus *Pseudomonas* Migula. The natural affinities of the representatives of this genus to those of the

genus *Vibrio*, as already pointed out by Migula, is so unmistakable that this amendment must be considered a decided improvement. Following the order of the *Eubacteriales* we encounter the order of the *Rhodobacteriales* comprising the natural group of the photosynthetic purple bacteria in two suborders: the sulfur-containing and the sulfur-free species. The merit of the demarcation of this order as compared with that of the *Thiobacteriales* Buchanan is to be found in the fact that the colourless sulfur bacteria of the genera *Beggiatoa*, *Thiothrix* and *Thioploca*, which show so different natural affinities (colourless *Cyanophyceae* as rightly suggested by Pringsheim), are not mixed up with the purple bacteria. Characteristic for Pringsheim's order of the *Mycobacteriales* is that it is again restricted to the homogeneous group formed by the genera *Corynebacterium*, *Mycobacterium* and *Actinomyces*. Significant for an appreciation of the clear insight of the author is his suggestion that the rod-shaped lactic acid bacteria might also belong to this order. The fifth order of the *Desmobacteriales* needs no comment since Pringsheim himself stresses its provisional nature.

From all the systems of classification based mainly on the morphology of the organisms Pringsheim's scheme is the most satisfactory one, and it compares especially favourably with Enderlein's [1925] revolutionary attempt at classifying the bacteria according to principles of comparative morphology and ontogeny ('cyclogeny'). This author rejects all previous attempts at classification because they are not based on a careful and thorough cytological study and from a theoretical standpoint his plea for attaching predominant importance to cytological and ontogenetic characters is very convincing. However, as pointed out earlier in this paper, our knowledge of characters of this kind is necessarily extremely limited and Enderlein's own contributions in this field do not change this situation to any appreciable extent. For it has escaped the attention of this zoologist that by far the greater part of the cytological details reported by him are theoretically undetectable since the dimensions of the structures described are below the limits of the resolving power of the microscope. This implies that most of the 'life-cycles' which are at the basis of Enderlein's system are fully artificial.* However tempting the classification outlined may be at first sight (*cf. e.g.* the table on p. 236), its value must be

* For sound ideas concerning the term 'life-cycle' the reader is referred to the note by Ch.-E. A. Winslow, *Science*, 81, 314, 1935.

judged from its results. A system which unites in one genus *Proteus vulgaris* and *Lactobacillus delbrückii*, and assumes close relationships of these with *Acetobacter*, *Rhizobium* (all in one and the same subfamily of the *Eisenbergiinae*) and with *Sclerothrix* (*Mycobacterium tuberculosis*) and *Corynebacterium* by placing all these genera in one of the 15 families, condemns itself.

Janke [1926], who in 1926 had already published a critical essay on the subject of bacterial systematics, later developed a system which is clearly influenced by Enderlein's views [Janke, 1929]. It seems superfluous to give here a complete survey of Janke's system. We will only remark that we encounter in this system many well-known groups – to which Janke assigns the rank of families – viz., *Coccaceae*, *Bacillaceae*, *Bacteriaceae*, *Corynebacteriaceae*, *Spirillaceae*, *Spirochaetaceae*, *Desmobacteriaceae* and *Myxobacteriaceae*. A discussion of the question whether the establishment of these units as families is more or less justified may be omitted here. Our remarks will be confined to the subdivision of the families. Janke's critical attitude towards the use of physiological characters for classificatory purposes leads him to restrict his genera to those groups which can be identified with the aid of morphological characters only. This means that e.g. in the family of the *Bacillaceae* the common aerobic and anaerobic sporeforming bacteria are united in the one genus *Bacillus* thus rejecting the genus *Clostridium* of various older systems. Moreover, many bacteriologists will be shocked to meet with the genus *Azotobacter* in this family. The justification of this procedure is found in the statement: 'Sporenbildende Stäbchen gehören zum Entwicklungskreis (Löhnis)'. The rather doubtful observations of Löhnis have, however, never been corroborated. It is also worth mentioning that the external morphology which is responsible for the maintenance of a separate genus *Azotobacter* in Janke's system is frequently nearly duplicated in species closely related to *Bacillus megaterium* [Dianowa and Woroschilowa, 1931] which is accepted by this author as a type species for a subgroup of the genus *Bacillus*.

The trend of thought mentioned above also accounts for the occurrence of only two genera, *Bacterium* and *Fusiformis*, in the family *Bacteriaceae*. The weakness of Janke's principles of classification is clearly evidenced by the heterogeneity of the genus *Bacterium*. The diversity of organisms collected by Enderlein in the subfamily of the *Eisenbergiinae*

— which has already been criticized above — is found here in one and the same division of the genus *Bacterium*.

The 10 contributions by Rahn [Rahn, 1929] and collaborators [Rahn, Laubengeyer and Mansfield, 1929] to the classification of bacteria will be mentioned here firstly for their very sound, meritorious criticisms of Bergey's system. For these we refer to the original papers and we will only remark that it is most discouraging that since their publication two new editions of Bergey's manual have appeared from which it is evident that the editing committee did not pay any heed to Rahn's amply documented considerations. In addition to calling attention to various fallacies in Bergey's system Rahn has also given a number of constructive suggestions, the most important of which will be briefly reviewed here. In the first place Rahn and Laubengeyer point out the close relationship between several of the *Bacteroides* species and the *Lactobacilli* and propose to abandon the illdefined genus by incorporating these species in the genus *Lactobacillus*, whilst grouping the remaining quite dissimilar species with some other genera. Rahn and Mansfield then show the desirability and the feasibility of collecting all polar flagellates out of the family of the *Bacteriaceae* into a separate family *Pseudomonadaceae* as had already been proposed by Winslow *et al.* in 1917. Finally there is a plea by Rahn for a due recognition of the intimate relation between the *Streptococci* and the natural group of the rod-shaped lactic acid bacteria. Rahn goes so far as to advocate the removal of the tribe *Streptococceae* from the family of the *Coccaceae* and its inclusion in the *Bacteriaceae*.

The most elaborate effort to revolutionize bacterial taxonomy in later years is undoubtedly the monograph published by Pribram [1933], an extension of the ideas expressed in an earlier paper [Pribram, 1929]. The most radical principle introduced is the division of the bacteria into three subclasses which are based chiefly on ecological considerations. Thus the subclass *Algobacteria* is meant to comprise all the forms adapted to a life in water, as evidenced a.o. by the motility with the aid of polar flagella. The second subclass, *Eubacteria*, is made up of those bacteria whose habitat is the animal body or complex waste products of plant or animal origin. This habitat has led to forms which are either immotile or motile by means of peritrichous flagella and which are characterized by their ability to attack complex molecules. Finally the third subclass, *Mycobacteria*, is adapted to a life in soil and

shows a distinct tendency to differentiation as manifested by the occurrence of spore formation, branching, etc.

Now the foundation of a classification chiefly on ecologic principles is undoubtedly very dangerous, as is clearly demonstrated by the obvious impossibility to apply such a scheme to the classification of higher organisms, where a more scientific taxonomy can be achieved on the basis of characters which have an undisputably phylogenetic value. It is therefore not surprising that a closer inspection of Pribram's system reveals its inadequacy. Thus, in order to defend the motility of a bacterium as a typical feature of an aquatic habitat Pribram has to resort to the enormity that the occurrence of peritrichous flagella amongst the representatives of the third group does not conflict with their supposed 'sessile' character, since here the flagella do not serve the purpose of locomotion but just act as organs to replenish the food supply.*

Moreover we find amongst the *Algobacteria* numerous species which are immotile, as well as organisms like various *Micrococcus* and *Sarcina* species which according to their habitat belong to the second subclass. And how can the author justify the inclusion of the peritrichous genera *Serratia* (and *Hillhousia*?) in the first subclass? The same holds, although for different reasons, for the genera *Myxococcus*, *Chondromyces* and *Polyangium*. The dissemination of a natural group like the *Thiorhodaceae* over three orders should not remain without protest and no investigator of this group of organisms will tolerate the placing of *Rhabdomonas* (N.B. as type genus) between the genera *Beggiatoa* and *Thioploca* and this merely on the basis of an alleged contractility of the cells.

Our criticisms of the second subclass are based for the greater part on the extreme heterogeneity of its constituent units. As for the second family our objections are substantially the same as those which have been brought forward in connexion with our review of Enderlein's subfamily of the *Eisenbergiinae*. We might remark in this connexion that all motile acetic acid bacteria (*Ulvina* spec.) which have been studied with respect to the mode of insertion of flagella have proved to be polar. In consequence their inclusion in the subclass *Eubacteria* conflicts with the first mentioned character in the diagnosis of this

* We have found the same critical remark in the attractive review of Pribram's monograph by C. Stapp, Zentralbl. Bakt. Parasitenk. Abt. II, 89, 514, 1934.

group. The objections raised by Rahn with respect to the genus *Bacteroides* apply also to the third family *Bacteroidaceae*.

The attempt to unite in one subclass those bacteria which show a tendency towards modes of reproduction other than fission is not objectionable in itself. However, the marked differences between the two orders makes it preferable to look upon these subgroups as terminal stages in the development of entirely different morphological groups and therefore, in our opinion, they should be ranged separately with their simpler ancestries. The advisability of the addition of several Gram-negative genera to the otherwise well characterized group of the *Mycobacteriales* seems also questionable.

The preceding remarks will suffice to make it clear that, although Pribram's genera are homogeneous and much better characterized than many of the genera adopted by Bergey *et al.*, we cannot approve of the way in which they are arranged in larger units. Which factors can be made responsible for this difference in appreciation? There can be no doubt that this is chiefly due to a different evaluation of the importance of various characters. Although Pribram pretends to attach a distinct value to the type of flagellation, yet the exceptions he allows in the consistent application of this diagnostic character are so numerous that already on this account a great deal of confusion results. Pribram's neglect of the suggestion made by Orla-Jensen that one should discern between 'incidental' and 'genuine' immotility – as discussed earlier – is another disturbing factor. Finally it is the insufficient appreciation of the importance of the Gram-reaction which should be criticized. On the one hand Pribram fully recognizes the value of this character which even accounts for the creation of several new genera. On the other hand the author fails to realize the obvious mutual affinities between many of the Gram-positive and many of the Gram-negative genera respectively. Perhaps it is more correct to say that these mutual relationships do not fully escape the attention of the author, since in various places mention is made of the fact that certain species may be considered as connecting links between two often widely separated groups. Our principal grievance against Pribram's classification is that such considerations have remained without any influence on the final form of his system.

The last contribution to bacterial classification is the important paper by Prévot [1933] which has already been quoted. This study is

especially attractive because the author starts with a clear formulation of the rules, principles and laws which, according to his insight, should govern bacterial systematics.

Prévot's first law states that the general morphology enables one to divide the bacterial kingdom in classes and these in orders. As classes Prévot recognizes the six orders previously established by Buchanan and a preliminary attempt is made at subdividing the first class (*Eubacteriales*) in five orders which apparently correspond to the five families of the American system. There is, however, the important difference that Prévot envisages the possibility of demarcating the *Nitrobacteriaceae* on the basis of an ellipsoidal cell form. Apart from the fact that our criticism of the said family as defined in the American system is not obviated by Prévot's redefinition, it seems to us very doubtful whether a distinction between two groups of the fundamental significance of an order can be founded on the difference between an ellipsoidal and a cylindrical cell shape. The failure of such a procedure is obvious if it is remembered that some of the most typical representatives of the *Bacteriaceae* have originally been described as *Micrococcus* species (*Micrococcus prodigiosus*, etc.). The ambiguity of the said character has, already a long time ago, led to abandoning Cohn's differentiation of the genera *Bacterium* and *Bacillus* on the basis of the length of the rods.

We can pass the second law in silence. As for the third law we welcome the stress laid on the importance of the Gram-stain, especially since good arguments are given for the view that the outcome of this staining reaction depends on structural characters. The homogeneity of a family in this respect is required by Prévot.

The fourth law formulates that more special morphological properties (flagella, capsules, biometrical constants) have a generic value. Why the use of these features should be restricted to these smaller groups is not clearly stated, however. To us this limitation does not seem justified particularly with reference to the flagellation.

The remaining laws do not invite further comments; we only refer to our opposition to the sixth law in which Prévot restricts the use of physiological characters to the delimitation of species.

Prévot has confined himself to apply the above-mentioned principles to the elaboration of a detailed classification of the *Coccaceae*. The outstanding feature of his system as compared with previous ones is the

early separation of the Gram-negative and the Gram-positive cocci, which ultimately leads to the creation of the new Gram-negative genus *Veillonella* which is the counterpart of the Gram-positive genus *Micrococcus*. In consequence of this the final system has the great merit of a logical and consistent structure. It should, however, be questioned whether it is commendable to restrict the physiological characters to specific delimitation. We can agree with the author that 'aerobic' and 'anaerobic' mode of life as based solely on the sensitivity of the organism towards free oxygen is a rather dangerous character. Yet it seems to us that the katabolic nature of the organism is a much more essential property and should not have been left out of consideration in the demarcation of higher groups than species. For it can hardly be doubted that the relationship between aerobic *Micrococcus* species and aerobic *Sarcina* species is at least as close as that between the latter and the anaerobic *Sarcina* species such as *Zymosarcina ventriculi* (Goodsir) Smit [Smit, 1930].

4. OUTLINE OF A RATIONAL SYSTEM FOR BACTERIAL CLASSIFICATION ON THE BASIS OF OUR PRESENT KNOWLEDGE

The preceding pages have not only served the purpose of exposing the manifold weaknesses and inconsistencies of the more recent systems, they may also perform the function of illustrating the considerations to which the general principles laid down in Section 2 lead in concrete instances. Therefore in the following attempt at sketching an outline of a rational bacterial system we shall also draw upon material which has already been used in the discussion of our attitude towards previous classifications.

It may be recalled that in the foregoing we have had the opportunity to point out that the assumption of both a morphological and a physiological evolution seems justified. A true reconstruction of the course of evolution is the ideal of every taxonomist. But, as has been rightly emphasized by Pringsheim, such a reconstruction is not feasible, firstly because the accepted relationships remain conjectural, and secondly because a number of the connecting links will be missing in the bacterial kingdom as it exists to-day. With Pringsheim we are of the opinion that 'ein wirklich wissenschaftliches System, das also mit Kritik aufgestellt wäre, gar nichts anderes tun könnte, als zu ver-

suchen, alle gegenseitigen Beziehungen der Organismen, die auf irgendeine Verwandtschaft hindeuten, wiederzugeben'.

The practical application of this line of thought obliges us to face the question whether in the construction of the system morphological or physiological characters should have priority in the expression of relationships between those systematic groups which are no longer morphologically and physiologically homogeneous.

In our opinion the binomial nature of nomenclature accentuates the demand that genera be systematic units which are characterized as well by a more or less complete morphological homogeneity as by a fundamental agreement in metabolic properties. This means, of course, that for the distinction of species within a genus only characters of secondary importance, such as biometric constants, hydrolytic abilities, the occurrence of pigments not determining the metabolic type, etc. can be applied.

Consequently the question raised above must be answered in order to make possible an arrangement of the genera in such a way as to satisfy best the natural relationships existing between them.

We have decided upon the use of morphological criteria as main guiding principle in the creation of systematic units above the rank of genera. In doing so we are fully aware that this choice may appear arbitrary. The resulting system unintentionally suggests that a morphological evolution has been primary and that in the various stages of morphological development an independent, though sometimes parallel, physiological differentiation has occurred afterwards. Yet it does not seem excluded at all that in special cases the order of events has been the reverse and that in reality parallel morphological evolutions have taken place in two physiologically different groups.

There is however, only a limited number of examples in which morphological differentiation in a clearly defined physiological group strongly suggests itself, whereas the instances are numerous that a typical katabolic process is found in groups morphologically so unrelated that affinities on the basis of physiology seem fully incompatible with the evolutionary idea. Moreover, in the former case the range of morphological differentiation extends but over a small number of closely related morphological units, as is *e.g.* clearly shown by the groups of purple bacteria. Therefore in a mainly morphological system such physiologically related groups will remain together, whilst, on

the other hand, in a mainly physiological system morphologically related groups will be widely dispersed.

Guided by these general considerations we shall first of all give a survey of the morphological units which can be distinguished in the bacterial kingdom and of the way in which they seem to be mutually related. This will be followed by the differentiation of the morphologically homogeneous groups on the basis of katabolism.

It seems acceptable that the diversity of bacterial forms is the outcome of various independent morphological evolutions which have had their starting-point in the simplest form both existent and conceivable: the sphere.

The existing forms suggest that four such evolutionary lines have to be distinguished.

a. In the first place we can observe that certain bacteria in which the spherical form is still fully maintained display a tendency to form complexes of a more or less regular appearance. Whereas little significance can be attached to the diplococcus form since its occurrence is inevitable in the reproduction process of the coccus, the formation of chains of four or more cocci is typical for several species. Obviously in this case one direction is preferential in cell division, the causative polarity becoming manifest owing to the fact that the cells remain attached.

In those spherical bacteria in which division takes place in two directions which meet at right angles complexes may originate which possess the typical form of tetrads or tetracocci. If finally the division occurs in three directions of space the outcome may be the formation of regular packets or *sarcinae*.

It must be emphasized, however, that the mentioned morphological groups comprise several transitional forms so that their delimitation, although desirable, is subject to many difficulties.

The occurrence of both Gram-positive and Gram-negative representatives among the coccoid forms indicates a micromorphological evolution which, although its direction cannot be traced, justifies a differentiation according to this character.

The highest developmental stage in the group of spherical organisms is in all probability displayed by the cocci able to form endospores, the existence of which has recently been firmly established by an investigation of Gibson [1935].

b. Motility occurs only sporadically among the cocci, yet it in-

dicates that also the flagellated rods find their origin in this primitive group, the more so since the two typical modes of flagellation are already encountered.

It seems only logical that the short Gram-negative polarly flagellated rods, as for instance characteristic for the species of the genus *Pseudomonas*, owe their origin to a Gram-negative monoflagellated coccus.* The polarity which arises from the attachment of a single flagellum to the sphere may easily induce a polar deviation in the form of the bacterium. In an analogous way the type of motility characteristic for polarly flagellated organisms, *viz.*, the rotation of the cell according to its longitudinal axis, may well be responsible for a gradual deformation of the cell resulting in the comma or vibrio form. The numerous

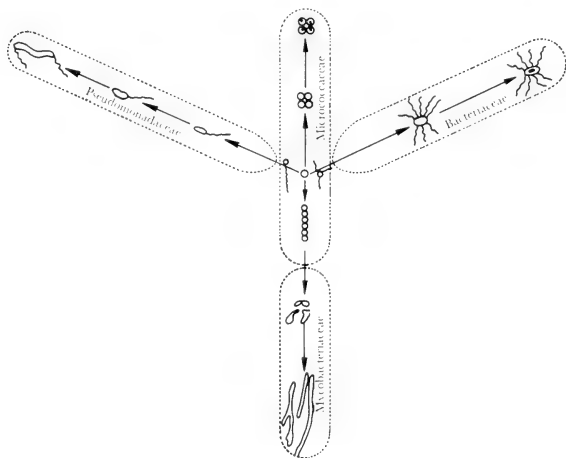


Fig. 1.

transitional forms between this morphological type and the spirillum forms leave no doubt as to the origin of the latter group.

c. The appearance of more than one flagellum distributed over the

* The same argumentation holds, of course, for the evolution of polarly flagellated Gram-positive rods (the newly created genus *Listerella*) in relation to a motile Gram-positive coccus.

surface of a coccus may well have been the starting-point for the development of motile rods with peritrichous flagella. As a result of this the representatives of the Gram-negative colon group (in its widest sense) and of the Gram-positive genus *Kurthia* would have arisen. It lies at hand that the endospore forming rods with peritrichous flagella represent a higher stage of development of these groups.

d. The fourth line of morphological evolution of the spherical cells seems to lead via the streptococci to the short Gram-positive rods in the group of lactic acid bacteria and corynebacteria. The further development of these universally immotile bacteria can have given rise to the mycobacteria which apparently form the connecting link with the simpler actinomycetes.

The various stages of these four independent evolutionary trends are represented in the following diagram (Fig. 1, see p. 307).

It appears appropriate to assign the rank of a family to each one of the four domains described and the names *Micrococcaceae*, *Pseudomonadaceae*, *Bacteriaceae* and *Mycobacteriaceae* are indicated.

The various morphological groups distinguished in each family will be designated as tribes. In accordance herewith the following tribes will be recognized:

Family	Tribes
<i>Micrococcaceae</i>	<i>Micrococceae</i>
	<i>Streptococceae</i>
	<i>Sarcineae</i>
	<i>Sporosarcineae</i>
<i>Pseudomonadaceae</i>	<i>Pseudomonadeae</i>
	<i>Vibrioneae</i>
	<i>Spirilleae</i>
<i>Bacteriaceae</i>	<i>Bacterieae</i>
	<i>Bacilleae</i>
<i>Mycobacteriaceae</i>	<i>Corynebacterieae</i>
	<i>Mycobacterieae</i>

Next we have listed the known, well distinguishable types of katabolism and so classified the bacteria answering the morphological requirements of the tribe according to their characteristic type of katabolism. As has already been argued organisms which on the basis of this procedure are collected in one and the same group should together constitute a genus.

In several instances this classification does not involve any difficulty, because the energetic requirements of many bacteria can be met by only one type of katabolic reaction. There are, however, also numerous cases in which it is clear that the organism can derive its energy from two or even more clearly distinct types of katabolism. This holds *e.g.* for the so-called facultatively anaerobic bacteria which in the presence of air are characterized by an oxidative katabolism (respiration), but which, under anaerobic conditions, depend upon some special type of fermentation. In those cases it is, of course, desirable to classify the organism in question according to its most characteristic type of katabolism, that is the type which permits the distinction from otherwise related organisms. This implies that for organisms capable of development under anaerobic conditions the katabolic process involved in this mode of life has been determinative, regardless of the question whether or not the organism also possesses a respiratory mechanism. If two different types of anaerobic katabolism, *e.g.* saccharolytic and proteolytic, are represented, the latter, as being the rarer, has been decisive.

In this way the system of classification represented in table I has been obtained.

The principles underlying this system would logically imply the creation of new generic names for all actually occurring combinations of fundamental morphological characters and special katabolic types. Experience shows, however, that in the majority of cases the natural groups obtained in this way coincide in all major points with various genera recognized in the systems now in use, at the same time demonstrating that our classificatory principles have, more or less unconsciously, already been applied by many of our predecessors. In all these cases we have, for practical reasons, maintained these current generic names, although naturally the generic diagnoses had to be amended more or less considerably.

In a few instances we have rejected a current generic name, although its diagnosis was sufficiently suited to justify its use. This was done because the generic name in question might give rise to confusion as a result of current nomenclature. Thus we have dropped the names *Thiobacillus* and *Rhodobacillus*, since these names wrongly suggest that they cover sporeforming bacteria. On analogous grounds we have used the generic name *Sulfospirillum* instead of *Thiospira*.

TABLE I. THE GENERA TO BE DISTINGUISHED IN THE VARIOUS

	Cephalotrichous (and related immotile) rod-shaped bacteria		
	Fam. <i>Pseudomonadaceae</i>		
	Spiral cells (Tribe <i>Spirillaceae</i>)	Curved rods (Tribe <i>Vibrionaceae</i>)	Straight rods (Tribe <i>Pseudomonadaceae</i>)
Photo-autotrophic:			
a. Bacteria with green pigment complex (Chlorobacteria)	—	—	—
b. Bacteria with purple pigment complex (<i>Thiorhodaceae</i>)	<i>Thiospirillum</i>	<i>Chromatium</i>	<i>Thiothece</i>
Photo-heterotrophic:			
a. Bacteria with brown pigment complex (Phaeobacteria)	<i>Phaeospirillum</i>	—	<i>Phaeomonas</i>
b. Bacteria with purple pigment complex (<i>Athiorhodaceae</i>)	<i>Rhodospirillum</i>	<i>Rhodovibrio</i>	<i>Rhodomonas</i>
Chemo-autotrophic:			
a. Bacteria which oxidize inorganic sulfur compounds (<i>Leucothiobacteria</i>)	<i>Sulfospirillum</i>	—	<i>Sulfomonas</i>
b. Bacteria which oxidize ferrous iron (and manganese)	—	<i>Didymohelix</i> ?	<i>Sideromonas</i>
c. Bacteria which oxidize ammonia	—	—	<i>Nitrosomonas</i>
d. Bacteria which oxidize nitrites	—	—	<i>Nitrobacter</i>
Chemo-heterotrophic:			
I. Bacteria with obligatory oxidative katabolism	<i>Spirillum</i>	<i>Vibrio</i>	<i>Acetobacter</i> <i>Pseudomonas</i> <i>Rhizobium</i> <i>Azotobacter</i> <i>Listerella</i> ?
II. Fermentative:			
a. 'Mixed acid' fermentation	—	—	—
b. Symmetric dimethylglycol (2,3-butylene- glycol) fermentation	—	—	<i>Aeromonas</i> ?
c. Alcoholic fermentation	—	—	<i>Zymomonas</i>
d. Butyric acid (butylalcohol and acetone) fermentation	—	—	—
e. Protein fermentation	—	—	—
f. Propionic acid fermentation	—	—	—
g. Homofermentative lactic acid fermentation	—	—	—
h. Heterofermentative lactic acid fermentation	—	—	—
i. Sulfate reduction	—	<i>Desulfovibrio</i>	—
k. Methane fermentation (CO ₂ -reduction)	—	—	<i>Methano- bacterium</i> ?

TRIBES ON THE BASIS OF KATABOLIC PROPERTIES

Spherical bacteria				Permanently immotile rod-shaped bacteria		Peritrichous (and related immotile) rod-shaped bacteria	
Fam. <i>Micrococcaceae</i>				Fam. <i>Mycobacteriaceae</i>		Fam. <i>Bacteriaceae</i>	
Cells single or in irregu- lar conglomerates (Tribe <i>Micrococcae</i>)	Cells in regular tetrads or packets (Tribe <i>Sarcinae</i>)	Cells in tetrads forming endospores (Tribe <i>Sporosarcinae</i>)	Cells in chains (Tribe <i>Streptococcae</i>)	Short rods (Tribe <i>Corynebacterieae</i>)	Long rods with a tenden- cy to form filaments (Tribe <i>Mycobacterieae</i>)	Non-sporeforming rods (Tribe <i>Bacterieae</i>)	Sporeforming rods (Tribe <i>Bacillae</i>)
<i>Chlorobium</i>	—	—	—	—	—	—	—
<i>Thiopoly- coccus</i>	<i>Thiopedia</i> <i>Thio- sarcina</i>	—	—	—	—	—	—
—	—	—	—	—	—	—	—
<i>Rhodococcus</i>	—	—	—	—	—	—	—
<i>Achro- matium</i>	—	—	—	—	—	—	—
<i>Siderocapsa</i>	—	—	—	—	—	—	—
<i>Nitrosococcus</i>	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
<i>Neisseria?</i> <i>Micrococcus</i>	<i>Gaffkya</i> <i>Sarcina</i>	<i>Sporo- sarcina</i>	—	<i>Coryne- bacterium</i>	<i>Mycobacterium</i>	<i>Kurthia</i> <i>Alcali- genes</i>	<i>Bacillus</i>
—	—	—	—	—	—	<i>Bacterium</i>	—
—	—	—	—	—	—	<i>Aerobacter</i>	<i>Aero- bacillus</i>
—	<i>Zymo- sarcina</i>	—	—	—	—	—	<i>Zymo- bacillus</i>
—	<i>Butyri- sarcina</i>	—	—	—	—	—	<i>Clostri- dium</i>
<i>Veillonella</i> <i>Peptococcus</i>	—	—	<i>Peptostrep- tococcus</i>	<i>Fusiformis</i> ?	—	—	<i>Peptoclos- tridium</i>
—	—	—	—	<i>Propioni- bacterium</i>	—	—	—
—	<i>Pedio- coccus</i>	—	<i>Strepto- coccus</i>	<i>Strepto- bacterium</i>	<i>Thermo- bacterium</i>	—	—
—	—	—	<i>Beta- coccus</i>	<i>Beta- bacterium</i>	—	—	—
—	—	—	—	—	—	—	—
<i>Methano- coccus</i>	<i>Methano- sarcina</i>	—	—	—	—	—	—

It is only self-evident that in a number of cases we have been obliged to establish new genera. The name *Desulfovibrio* for the sulfate reducing bacteria first described by Beijerinck and Van Delden needs no further comment. *Zymomonas* has been chosen as a suitable name for polarly flagellated bacteria causing alcoholic fermentation like *Pseudomonas lindneri* (*Termobacterium mobile* Lindner), *Aeromonas* for similar organisms which ferment sugars in a way probably closely related to the fermentation type characteristic of the genera *Aerobacter* and *Aerobacillus*. The genus *Methanobacterium* has been created for the methane producing rod-shaped bacteria described by Söhngen [1906]. Similarly *Methanosarcina* is a new generic name for the related sarcina, *Methanococcus* for the small spherical forms causing this type of fermentation [Groenewege, 1920]. *Peptococcus* and *Peptostreptococcus* represent the anaerobic proteolytic micrococci and streptococci respectively; *Butyrisarcina* has been adopted as a designation for the butyric acid producing *Zymosarcina maxima* (Lindner) Smit and possibly allied forms.

Finally the name *Zymobacillus* has been introduced for the facultatively anaerobic sporeforming bacteria of the type *Bacillus macerans* Schardinger.

Provisionally, and with great reservation, we have added two genera, *Phaeospirillum* and *Phaeomonas*, for bacteria belonging to the group of organisms which Utermöhl [1924] has designated as Phaeobacteria. These show very close affinities with the *Athiorhodaceae*, both morphologically and physiologically. It is entirely possible that a careful study of these brown bacteria will reveal facts which would justify their inclusion in the corresponding genera of the purple bacteria, *Rhodospirillum* and *Rhodomonas*.

In the composition of the table some difficulty was experienced, however, in view of the fact that for a few of the existing genera the katabolic properties are still insufficiently known. In some cases the relationships of such genera to organisms, whose position could be readily determined, were more or less evident, and therefore their place in the table has tentatively been indicated. This holds *e.g.* for the genera *Aeromonas* and *Fusiformis*. In other cases even such indications are lacking, and so a few genera well-known to the medical bacteriologist could not yet be classified. This applies *e.g.* to *Pasteurella*, *Dialister*, *Haemophilus*, *Listerella* and to the species *Corynebacterium diphtheriae* which, owing to its indubitable fermenting capacity, cannot be

grouped with the obligatory oxidative saprophytic corynebacteria.

For analogous reasons, but now because of the scantiness of morphological data, the position of the genera *Nitrobacter*, *Didymohelix*, *Sideromonas*, *Kurthia*, and *Methanobacterium* must be deemed provisional.

The preliminary nature of the table manifests itself also by the circumstance that in a restricted number of cases it was considered desirable to maintain a number of genera which according to the principle adopted should have been amalgamated.

Because we have felt that there possibly exist sufficiently essential differences between such genera as *Azotobacter*, *Acetobacter*, *Kurthia*, and the genera with which they are grouped, we have refrained from rashly abolishing them, although the morphological differentiation which is at the basis of our system does not suffice to keep them separated. In some instances a separation is already fully justified on the basis of the outcome of the Gram-stain. In this connexion we refer to the genera *Kurthia-Alcaligenes*, and *Listerella-Pseudomonas*, the species of the first-mentioned genus of each couple being Gram-positive, those of the second one Gram-negative. We may trust, moreover, that future investigations will provide the means for a more adequate generic differentiation in the cases under discussion.

When we now proceed to an examination of the table as a whole, we must first of all realize that its two-dimensional nature evidently does not permit of a true representation of the natural relationships of the various genera. The postulated many-sided morphological evolution starting from the group of spherical organisms in reality asks for a grouping of the three families of rod-shaped organisms around that of the *Micrococcaceae*.

Notwithstanding this shortcoming of the table it is obvious that in many of the physiological groups distinguished the distribution of the genera is far from random, as a glance at the upper half of the table shows convincingly. Apparently there is a close correlation between the phototrophic and chemo-autotrophic modes of life and morphology. In this domain it is therefore rather inessential whether a physiological or a morphological evolution is assumed to have been primary. The mutual relationships remain clearly expressed in either case. Another physiological group, *viz.*, that of the true lactic acid bacteria, also shows a definite correlation between physiological and morphological properties.

A further striking feature of the table is undoubtedly the occurrence of an obligatory oxidative metabolism in nearly all morphological types. Although this is partly due to the fact that in the present state of our knowledge it is impossible to carry through a subdivision of the aerobic metabolism into different types in a way comparable with that which has been applied in the case of fermentative metabolism, yet the table suggests that in the group of the obligatory oxidative heterotrophic bacteria the main morphological evolution has occurred. In this line of thought the physiological evolution will have taken place in the different stages of this primary development. In this connexion it must be remembered that in several cases the fermentative metabolism is only a supplement to the oxidative mode of life. The assumption made would also account for the more or less haphazard distribution of the various katabolic types in the different morphological units.

A few words remain to be said with regard to the distribution of the Gram-positive and Gram-negative genera in the system. As has been remarked before we attach sufficient importance to this character to require that a genus be homogeneous in this respect. Yet it appears that the system given does not result in a clear-cut separation of the Gram-positive and the Gram-negative genera. For the family of the *Micrococcaceae* this differentiation has already been remarked upon. In the family of the *Pseudomonadaceae* Gram-negative genera are strongly predominant, whereas on the contrary in the family of the *Mycobacteriaceae* only Gram-positive organisms are encountered. Finally it does not seem excluded that in the family of the *Bacteriaceae* a parallel development has taken place in both Gram-positive and Gram-negative forms. If the reported occurrence of Gram-negative *Bacillus* species should be confirmed a division of this genus will become inevitable which might lead to a clarification of the affinities in the family as a whole.

It will not have escaped attention that in the foregoing most 'bacteria' which, already in previous systems, have been separated from the so-called *Eubacteriales* have been left out of consideration. This holds for the *Spirochaetales*, the *Myxobacteriales*, the *Actinomycetales* (p.p.) and the *Chlamydobacteriales*. The *Thiobacteriales* in the sense of Buchanan have been included with the exception of the genera *Beggiatoa*, *Thiothrix* and *Thioploca*. The representatives of the latter genera show such

unmistakable morphological affinities with the *Cyanophyceae* that in our opinion they must be considered as colourless derivatives of the genera *Oscillatoria*, *Phormidium* and *Schizothrix* (see also Pringsheim l.c.).

As for the *Spirochaetales* and the *Myxobacterales* they form well-defined groups whose affinities with the *Eubacterales* are at least doubtful. Therefore they may well be ignored here. On the other hand the relationship of the Actinomycetes to the *Eubacterales* is very clear; as is generally accepted they represent stages of higher development of the *Mycobacterieae*. At the same time they form such a special and extensive group by themselves that their classification falls outside the scope of this study.

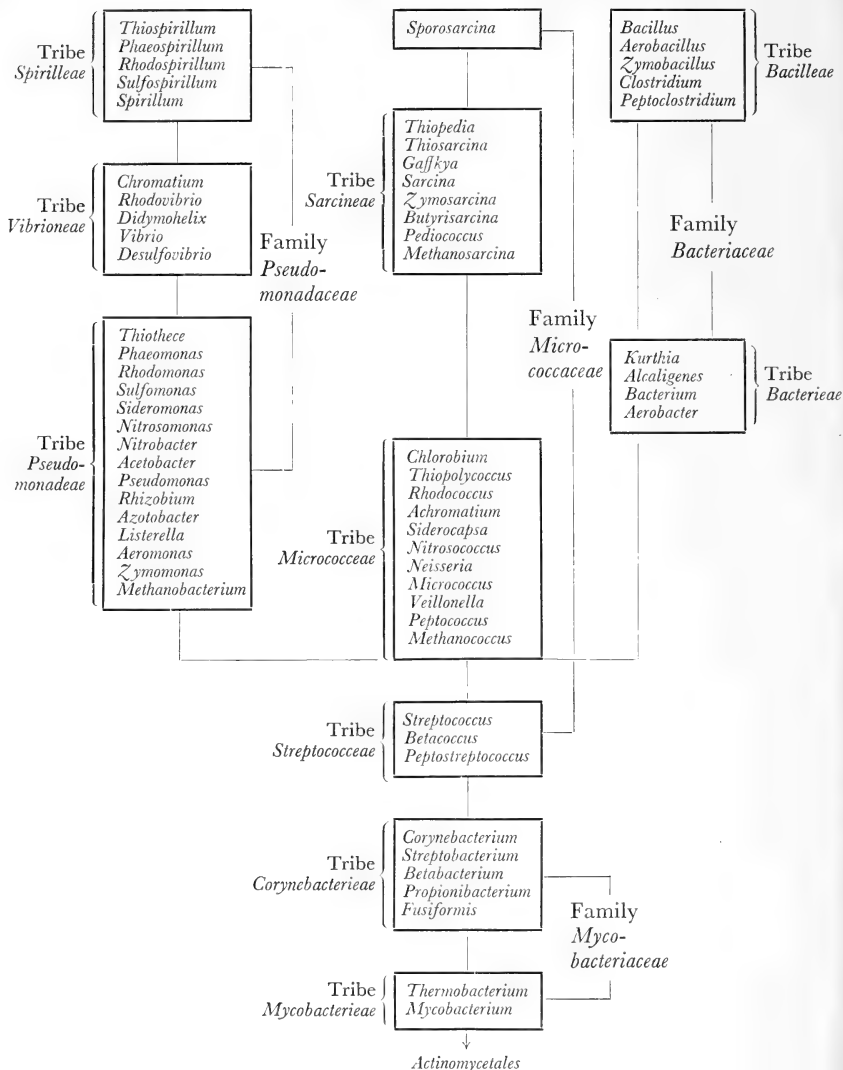
The remaining order of the *Chlamydobacterales* cannot so easily be disposed of. At first sight the representatives of genera like *Leptothrix*, *Crenothrix* and *Sphaerotilus* do not seem at all related to any of the *Eubacterales*. Although we cannot yet express any definite opinion regarding the taxonomy of this group it appears probable that it is very heterogeneous and that it harbours organisms (*Crenothrix*!) which are closely related or belong to the *Eumycetes*, as well as organisms which, like *Sphaerotilus natans*, are related to sheath-free filamentous *Eubacterales* (*Bacillus*, *Mycobacterium* and *Thermobacterium* species). In this respect it is tempting to draw the attention on the one hand to such organisms as *Leptothrix hyalina* (Migula) Bergey *et al.* and *Sphaerotilus paludosus* Smit [Smit, 1934] which, although they are usually reckoned to belong to the *Chlamydobacterales*, are reported to lack a sheath, and on the other hand to a bacterium, like *Bacillus funicularius*, which, although a true *Bacillus* species, forms a distinct sheath under special nutritional conditions [Kluyver and Van Niel, 1926]. Moreover, the latter phenomenon is also frequently encountered in the group of the true lactic acid bacteria [Orla-Jensen, 1919].

Table II gives a survey of the families, tribes and genera of the order *Eubacterales*. After all that has already been said this table will not need any further comment.

In concluding we wish to make the following remarks.

As has been amply set forth in the discussion of the general principles of classification it is necessary both from a scientific and from a practical standpoint to aim at a system which is worthy of the designation 'natural'. We fully realize that the result of our own classificatory attempt has only very imperfectly approximated this goal.

TABLE II. THE FAMILIES, TRIBES, AND GENERA OF THE EUBACTERIALES



On the other hand it seems to us that the system as outlined in this paper marks a definite progress as compared with previous ones. To justify this statement it will suffice to bring out two features.

Firstly the system given is characterized by its simplicity as well as by its consistency. The principle that a genus in all cases is defined by the fundamental morphological and katabolic properties makes for units which are much more easily distinguishable than the majority of the genera in the previous systems. In addition the system offers the advantage of permitting the ready incorporation of organisms with still unknown combinations of morphological and physiological characters. Therefore it may lay claim to the epithet 'rational'.

But secondly it seems to us that to a certain extent the system, although artificial, also answers the requirements of a true natural system. For the natural relationships between the various bacteria which in the present state of our knowledge can be vaguely perceived find a definite expression in the aspect of the system. That this aspect is not final is certain. Future investigations will deepen our insight into the natural relationships of different bacterial groups and the system will have to be modified accordingly.

Nevertheless it appears likely that the idea which is largely responsible for the outline, *viz.*, the occurrence of both morphological and katabolic evolution in the bacterial kingdom, will reappear in future classifications, thus perhaps justifying the use of the word prospects in the title of this paper. In the meantime the system in its present imperfect shape may well serve the purpose of stimulating interest and research in this field.

APPENDIX

LIST OF GENERA INCLUDED IN THE TABLES
AND THEIR DIAGNOSIS*I. Tribe *Spirilleae*

1. *Thiospirillum* Winogradsky, 1888.
Spiral bacteria, motile by means of cephalotrichous flagella. No endospores formed. Photo-autotrophic, containing a red to purple pigment complex. Normally reducing carbon dioxide with the simultaneous oxidation of H_2S or other inorganic sulfur compounds.
The type species is *Thiospirillum sanguineum* (Ehrenberg) Winogradsky.
2. *Phaeospirillum* nov. gen.
Spiral bacteria, motile by means of cephalotrichous flagella. No endospores formed. Photo-heterotrophic, containing a brown pigment complex.
The type species to be assigned in the near future.
3. *Rhodospirillum* Molisch, 1907.
Spiral bacteria, motile by means of cephalotrichous flagella. No endospores formed. Photo-heterotrophic, containing a red to purple pigment complex.
The type species is *Rhodospirillum rubrum* (Esmarch) Molisch.
4. *Sulfospirillum* nov. gen. Syn.: *Thiospira* Wislouch, 1914.
Spiral bacteria, motile by means of cephalotrichous flagella. No endospores formed. Chemo-autotrophic, oxidizing H_2S or other inorganic sulfur compounds.
The type species is *Sulfospirillum winogradskyi* (Omelianski).
5. *Spirillum* Ehrenberg, 1830.
Spiral bacteria, motile by means of cephalotrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram negative.
The type species is *Spirillum undula* (Müller) Ehrenberg.

* Although the diagnoses of those genera which have been retained from earlier systems of classification have nearly all been subject to more or less considerable amendments, we have indicated this only explicitly by the suffix 'Emend.' in those cases in which the amendment introduced involved the elimination of well established species from the genus.

Secondly it has to be emphasized that the following generic diagnoses have purposely been kept broad, although they might, in most cases, well have been elaborated on the basis of the available information concerning the representatives described until now. We have refrained from this in order to permit the future incorporation of species which deviate in minor respects from the collectivity now included in the genus, but which, nevertheless, show the morphological features of the tribe, and the katabolic activity deemed characteristic of the genus.

II. Tribe *Vibrioneae*

1. *Chromatium* Perty, 1852.

Slightly curved rods, motile by means of cephalotrichous flagella. No endospores formed. Photo-autotrophic, containing a red to purple pigment complex. Normally reducing carbon dioxide with the simultaneous oxidation of H_2S or other inorganic sulfur compounds.

The type species is *Chromatium okenii* Perty.

2. *Rhodovibrio* Molisch, 1907.

Slightly curved rods, motile by means of cephalotrichous flagella. No endospores formed. Photo-heterotrophic, containing a red to purple pigment complex.

The type species is *Rhodovibrio parvus* Molisch.

3. *Didymohelix* Griffith, 1853. Syn.: *Gallionella* Ehrenberg, 1838.

Curved rods, motile (?). No endospores formed. Chemo-autotrophic, oxidizing ferrous iron. The ferric hydroxide is deposited in the form of a twisted band which carries the organism at the top.

The type species is *Didymohelix ferruginea* (Ehrenberg) Griffith.

4. *Vibrio* Müller, 1773.

Curved rods, motile by means of cephalotrichous flagella. Occasionally spiral forms are present. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-negative.

The type species is *Vibrio comma* (Schroeter) Bergey *et al.*

5. *Desulfovibrio* nov. gen.

Curved rods, motile by means of cephalotrichous flagella. Occasionally spiral forms are present. No endospores formed. Chemo-heterotrophic, anaerobic, oxidize organic substances with the simultaneous reduction of sulfate to sulfide.

The type species is *Desulfovibrio desulfuricans* (Beijerinck), Syn.: *Spirillum desulfuricans* Beijerinck.

III. Tribe *Pseudomonadeae*

1. *Thiothece* Winogradsky, 1888*.

Ellipsoidal to rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Photo-autotrophic, containing a red to purple pigment complex. Normally reducing carbon dioxide with the simultaneous oxidation of H_2S or other inorganic sulfur compounds.

The type species is *Thiothece gelatinosa* Winogradsky.

* It seems highly doubtful whether the other genera created by Winogradsky for similarly shaped organisms (*Thiocystis*, *Lamprocystis*, *Amoebobacter*, *Thiodictyon*) are sufficiently different to be maintained. Also various of the purple sulfur bacteria which have been described as *Chromatium* species should be reckoned to *Thiothece*.

2. *Phaeomonas* nov. gen.
Ellipsoidal to rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Photo-heterotrophic, containing a brown pigment complex.
The type species is *Phaeomonas varians* (Ewart), Syn.: *Streptococcus varians* Ewart.
3. *Rhodomonas* Orla-Jensen, 1909. Emend. (!)* Syn.: *Rhodobacillus* Molisch, 1907.
Ellipsoidal to rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Photo-heterotrophic, containing a red to purple pigment complex.
The type species is *Rhodomonas palustris* (Molisch), Syn.: *Rhodobacillus palustris* Molisch.
4. *Sulfomonas* nov. gen. Syn.: *Thiobacillus* Beijerinck, 1904.
Ellipsoidal to rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Chemo-autotrophic, oxidizing H_2S or other inorganic sulfur compounds.
The type species is *Sulfomonas thiopara* (Beijerinck), Syn.: *Thiobacillus thioparus* Beijerinck.
5. *Sideromonas* Cholodny, 1922.
Ellipsoidal to rod-shaped bacteria, immotile (?). No endospores formed. Chemo-autotrophic, oxidizing ferrous iron.
The type species is *Sideromonas confervaria* Cholodny.
6. *Nitrosomonas* Winogradsky, 1892.
Ellipsoidal to rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Chemo-autotrophic, oxidizing ammonia to nitrite.
The type species is *Nitrosomonas europaea* Winogradsky.
7. *Nitrobacter* Winogradsky, 1892.
Ellipsoidal to rod-shaped bacteria, either immotile or motile by means of cephalotrichous flagella. No endospores formed. Chemo-autotrophic, oxidizing nitrite to nitrate.
The type species is *Nitrobacter winogradskyi* Buchanan.
8. *Acetobacter* Beyerinck, 1900.
Ellipsoidal to rod-shaped bacteria, either immotile or motile by means of cephalotrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds, with a marked tendency to form acids as products of incomplete oxidation. Adapted to life in acid media.
The type species is *Acetobacter pasteurianum* (Hansen) Beijerinck.
9. *Pseudomonas* Migula, 1894.
Ellipsoidal to rod-shaped bacteria, either immotile or motile by means of

* It has to be clearly understood that the diagnosis of this genus as given above is not at all in agreement with the diagnosis given by Orla-Jensen who uses the name as a synonym for *Chromatium*. Nevertheless, the name in question is adopted here to match the names of the related genera *Rhodospirillum*, *Rhodovibrio* and *Rhodococcus*.

cephalotrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-negative. Adapted to life in neutral to slightly alkaline media.

The type species is *Pseudomonas fluorescens* (Flügge) Migula.

10. *Rhizobium* Frank, 1889.

Ellipsoidal to rod-shaped bacteria, motile by means of cephalotrichous flagella. Branched and swollen involution forms. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. The association of the bacteria and *Leguminosae* fixes atmospheric nitrogen (root-nodules!).

The type species is *Rhizobium radicicola* (Beijerinck).

11. *Azotobacter* Beijerinck, 1901.

Ellipsoidal to rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic substances. Capable of fixing atmospheric nitrogen.

The type species is *Azotobacter chroococcum* Beijerinck.

12. *Listerella* Pirie, 1927.

Short rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-positive.

The type species is *Listerella monocytogenes* (Murray *et al.*) Pirie.

13. *Aeromonas* nov. gen.

Short rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds, and capable of fermenting carbohydrates with the production of carbon dioxide and hydrogen, and 2,3-butylene glycol (?). Gram-negative. The type species is *Aeromonas liquefaciens* (Beijerinck), Syn.: *Aerobacter liquefaciens* Beijerinck.

14. *Zymomonas* nov. gen.

Ellipsoidal to rod-shaped bacteria, motile by means of cephalotrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds, and capable of fermenting carbohydrates with the production of carbon dioxide, ethyl alcohol, and lactic acid. Gram-negative.

The type species is *Zymomonas mobile* (Lindner), Syn.: *Termobacterium mobile* Lindner.

15. *Methanobacterium* nov. gen.

Straight or slightly bent rods, often united to bundles or long chains. Motility not observed. No endospores formed. Chemo-heterotrophic, anaerobic, fermenting various organic compounds with the formation of methane. Gram negative. The type species to be described in the near future.

IV. Tribe *Micrococceae*

1. *Chlorobium* Nadson, 1906.

Spherical cells with a tendency to the formation of chains, rods, and even filamentous forms, the latter sometimes wound in tight spirals. Motility has not

been observed. No endospores formed. Photo-autotrophic, containing a green pigment complex. Reduce carbon dioxide with the simultaneous oxidation of H_2S to sulfur.

The type species is *Chlorobium limicola* Nadson.

2. *Thioplycoccus* Winogradsky, 1888.

Spherical cells, usually occurring in irregular masses, motility has not been observed. No endospores formed. Photo-autotrophic, containing a red to purple pigment complex. Normally reducing carbon dioxide with the simultaneous oxidation of H_2S or other inorganic sulfur compounds.

The type species is *Thioplycoccus ruber* Winogradsky.

3. *Rhodococcus* Molisch, 1907 (non Zopf).

Spherical cells, motility has not been observed. No endospores formed. Photo-heterotrophic, containing a red to purple pigment complex.

The type species is *Rhodococcus capsulatus* Molisch.

4. *Achromatium* Schewiakoff, 1893*.

Large spherical cells, motile (no flagella?). No endospores formed. Chemo-autotrophic, oxidizing H_2S or other inorganic sulfur compounds.

The type species is *Achromatium oxaliferum* Schewiakoff.

5. *Siderocapsa* Molisch, 1909.

Spherical cells, motility has not been observed. No endospores formed. Chemo-autotrophic, oxidizing ferrous iron.

The type species is *Siderocapsa treubii* Molisch.

6. *Nitrosococcus* Winogradsky, 1892.

Spherical cells, motile by means of a single flagellum. No endospores formed. Chemo-autotrophic, oxidizing ammonia to nitrite.

The type species is *Nitrosococcus nitrosus* (Migula) Buchanan.

7. *Neisseria* Trevisan, 1885.

Spherical cells, normally occurring in flattened pairs, immotile. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-negative.

The type species is *Neisseria gonorrhoeae* Trevisan.

8. *Micrococcus* Cohn, 1872.**

Spherical cells, occurring singly, in pairs, or in irregular masses. Either motile or immotile. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-positive.

The type species is *Micrococcus luteus* (Schroeter) Cohn.

* It seems probable that the organisms described as *Hillhousia*, *Modderula*, *Thiophysa*, and *Thiovulum* species belong to this genus.

** There should, perhaps, be a genus containing Gram-negative oxidative cocci in addition to the genus *Neisseria*, the more so since the katabolism of representatives of this genus is insufficiently known. Although Prévot (l.c.) is inclined to include also aerobic Gram-negative species in his genus *Veillonella*, this generic name will be used by us to designate the corresponding anaerobic species only.

9. *Veillonella* Prévot, 1932. Emend.*

Spherical cells, motility has not been observed. No endospores formed. Chemo-heterotrophic, anaerobic, capable of fermenting protein decomposition products with the production of carbon dioxide, hydrogen, and other unknown products. Gram-negative.

The type species is *Veillonella parvula* (Veillon et Zuber) Prévot.

10. *Peptococcus* nov. gen.**

Spherical cells, either immotile or motile. No endospores formed. Chemo-heterotrophic, anaerobic, capable of fermenting protein decomposition products. Gram-positive.

The type species is *Peptococcus niger* (Hall).

11. *Methanococcus* nov. gen.

Spherical cells, occurring singly or in masses. Motility has not been observed. No endospores formed. Chemo-heterotrophic, anaerobic, fermenting various organic compounds with the formation of methane. Gram-negative (?).

The type species to be assigned in the near future.

V. Tribe *Sarcineae*

1. *Thiopedia* Winogradsky, 1888.

Spherical cells, occurring in regular tetrads which are often united to unicellular layers. Motility has not been observed. No endospores formed. Photo-autotrophic, containing a red to purple pigment complex. Normally reducing carbon dioxide with the simultaneous oxidation of H_2S or other inorganic sulfur compounds.

The type species is *Thiopedia rosea* Winogradsky.

2. *Thiosarcina* Winogradsky, 1888.

Spherical cells in regular packets. No endospores formed. Photo-autotrophic, containing a red to purple pigment complex. Normally reducing carbon dioxide with the simultaneous oxidation of H_2S or other inorganic sulfur compounds.

The type species is *Thiosarcina rosea* (Schroeter) Winogradsky.

3. *Gaffkya* Trevisan, 1885.

Spherical cells often occurring in regular tetrads. Motility has not been observed. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-positive.

The type species is *Gaffkya tetragena* (Gaffky) Trevisan.

4. *Sarcina* Goodsir, 1842.

Spherical cells, often occurring in regular packets. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-positive.

The type species is *Sarcina lutea* Schroeter.

* Cf. the previous note.

** The various anaerobic, Gram-positive cocci placed by Prévot (l.c.) in the genera *Diplococcus*, *Gaffkya*, *Staphylococcus* and *Micrococcus* might well be united in this genus, as long as further particulars regarding their katabolism are lacking.

5. *Zymosarcina* Smit, 1930.
Spherical cells, generally occurring in regular packets. No endospores formed. Chemo-heterotrophic, anaerobic, fermenting carbohydrates with the formation of ethyl alcohol and carbon dioxide. Gram-positive.
The type species is *Zymosarcina ventriculi* (Goodsir) Smit.
6. *Butyrisarcina* nov. gen.
Spherical cells, generally occurring in regular packets. No endospores formed. Chemo-heterotrophic, anaerobic, fermenting carbohydrates chiefly with the formation of butyric and acetic acids, carbon dioxide and hydrogen. Gram-positive.
The type species is *Butyrisarcina maxima* (Lindner).
7. *Pediococcus* Balcke 1884.
Spherical cells, generally occurring in tetrads. No endospores formed. Chemo-heterotrophic, fermenting carbohydrates exclusively to lactic acid. Gram-positive.
The type species is *Pediococcus damnosus* Claussen.
8. *Methanosarcina* nov. gen.
Spherical cells, generally occurring in regular packets. No endospores formed. Chemo-heterotrophic, anaerobic, fermenting various organic substances with the formation of methane. Gram-negative.
The type species is *Methanosarcina methanica* (Smit).

VI. Tribe *Sporosarcineae*

1. *Sporosarcina* Orla-Jensen, 1909.
Spherical cells, generally occurring in regular tetrads or packets. Either motile or immotile. Endospores are formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-negative.
The type species is *Sporosarcina ureae* (Beijerinck).

VII. Tribe *Streptococceae*

1. *Peptostreptococcus* nov. gen.
Spherical cells, generally occurring in chains. Immotile. No endospores formed. Chemo-heterotrophic, anaerobic, capable of fermenting protein decomposition products with the formation of carbon dioxide, hydrogen, and other unknown products. Gram-positive.
The type species is *Peptostreptococcus anaerobius* (Krönig et Menge).
2. *Streptococcus* Rosenbach, 1884. Emend. Orla-Jensen, 1919.
Spherical cells, generally occurring in chains. As a rule immotile. No endospores formed. Chemo-heterotrophic, fermenting carbohydrates with the practically exclusive formation of lactic acid. Gram-positive.
The type species is *Streptococcus lactis* (Lister) Löhnis.
3. *Betacoccus* Orla-Jensen, 1919.
Spherical cells, generally occurring in chains. Immotile. No endospores formed.

Chemo-heterotrophic, fermenting carbohydrates with the formation of lactic and acetic acid, ethyl alcohol and carbon dioxide. Gram-positive.
The type species is *Betacoccus arabinosaceus* Orla-Jensen.

VIII. Tribe *Corynebacterieae*

1. *Corynebacterium* Lehmann et Neumann, 1896. Emend. (!).
Cells ovoid to rod-shaped, the latter frequently swollen or branched. Immotile. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-positive.
The type species is *Corynebacterium pseudodiphtheriticum* Lehmann et Neumann.
2. *Fusiformis* Hoelling, 1910. Emend. (!).
Cells ovoid, frequently elongate and fusiform, filaments sometimes formed. Immotile. No endospores formed. Chemo-heterotrophic, fermenting protein decomposition products (?). Gram-positive (?).
The type species is *Fusiformis dentium* Hoelling.
3. *Propionibacterium* Orla-Jensen, 1909.
Cells ovoid to rod-shaped, the latter frequently swollen or branched. Immotile. No endospores formed. Chemo-heterotrophic, fermenting carbohydrates with the formation of propionic acid as the main product. Gram-positive.
The type species is *Propionibacterium jensenii* Van Niel.
4. *Streptobacterium* Orla-Jensen, 1919.
Cells ovoid to rod-shaped, frequently in chains. Immotile. No endospores formed. Chemo-heterotrophic, fermenting carbohydrates with the practically exclusive formation of lactic acid. Gram-positive.
The type species is *Streptobacterium casei* Orla-Jensen.
5. *Betabacterium* Orla-Jensen, 1919.
Cells ovoid to rod-shaped, frequently in chains. Immotile. No endospores formed. Chemo-heterotrophic, fermenting carbohydrates with the formation of lactic and acetic acid, ethyl alcohol and carbon dioxide. Gram-positive.
The type species is *Betabacterium breve* Orla-Jensen.

IX. Tribe *Mycobacterieae*

1. *Mycobacterium* Lehmann et Neumann, 1896.
Rod-shaped bacteria, with a marked tendency to the formation of branched and filamentous cells. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-positive, acid-fast.
The type species is *Mycobacterium tuberculosis* (Koch) Lehmann et Neumann.
2. *Thermobacterium* Orla-Jensen, 1919.
Rod-shaped bacteria, with a tendency to the formation of filamentous cells. No endospores formed. Chemo-heterotrophic, fermenting carbohydrates with the practically exclusive formation of lactic acid. Gram-positive.
The type species is *Thermobacterium cereale* Orla-Jensen.

X. Tribe *Bacterieae*1. *Kurthia* Trevisan, 1885.

Rod-shaped bacteria, sometimes united in chains. Either immotile or motile by means of peritrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-positive.

The type species is *Kurthia zoffii* (Kurth) Trevisan.

2. *Alcaligenes* Castellani et Chalmers, 1918.*

Rod-shaped bacteria. Either immotile or motile by means of peritrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-negative.

The type species is *Alcaligenes faecalis* Castellani et Chalmers.

3. *Bacterium* Ehrenberg, 1828. Emend. (!).**

Rod-shaped bacteria. Either immotile or motile by means of peritrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds, and capable of fermenting carbohydrates with the formation of various organic acids, and usually hydrogen and carbon dioxide. No acetylmethylcarbinol formed. Gram-negative.

The type species is *Bacterium coli* Escherich.

4. *Aerobacter* Beijerinck, 1900. Emend.***

Rod-shaped bacteria. Either immotile or motile by means of peritrichous flagella. No endospores formed. Chemo-heterotrophic, oxidizing various organic compounds, and capable of fermenting carbohydrates, 2,3-butylene glycol being one of the main products. Acetylmethylcarbinol is formed. Gram-negative.

The type species is *Aerobacter aerogenes* (Escherich) Beijerinck.

XI. Tribe *Bacilleae*1. *Bacillus* Cohn, 1872.

Rod-shaped bacteria, sometimes occurring in chains. Either immotile or motile by means of peritrichous flagella. Endospores formed. Chemo-heterotrophic, oxidizing various organic compounds. Gram-positive.

The type species is *Bacillus subtilis* (Ehrenberg) Cohn.

* Including the genus *Brucella*.

** It should be well understood that in this genus are included the genera *Proteus*, *Salmonella*, *Eberthella*, and *Shigella*, which nowadays are frequently used. Although specialists dealing with this group may feel the need of further subdivision, yet it is unacceptable to us to differentiate genera on such trifling characters as are used in the current systems. Two organisms which ferment glucose in essentially the same way should not, in our opinion, be placed in separate genera, because one of them ferments (*i.e.* hydrolyzes!) also lactose. Nor should this be done because one of them splits formic acid into carbon dioxide and hydrogen, whereas the other does not (*i.e.* acid but no gas produced!).

*** Including the genera *Serratia* and *Klebsiella*.

2. *Aerobacillus* Donker, 1926. Emend. (!).
Rod-shaped bacteria. Either immotile or motile by means of peritrichous flagella. Endospores formed. Chemo-heterotrophic, oxidizing various organic compounds, and capable of fermenting carbohydrates, 2,3-butylene glycol and ethyl alcohol being the main products. Gram-positive.
The type species is *Aerobacillus polymyxa* (Prazmowski) Donker.
3. *Zymobacillus* nov. gen.
Rod-shaped bacteria. Either immotile or motile by means of peritrichous flagella. Endospores formed. Chemo-heterotrophic, oxidizing various organic compounds, and capable of fermenting carbohydrates, ethyl alcohol, acetic acid and acetone being the main products. Gram-positive.
The type species is *Zymobacillus macerans* (Schardinger).
4. *Clostridium* Prazmowski, 1880.
Rod-shaped bacteria, sometimes occurring in chains. Either immotile or motile by means of peritrichous flagella. Endospores formed, as a rule in typical clostridia or plectridia. Chemo-heterotrophic, anaerobic, fermenting carbohydrates with the formation of butyric and acetic acid, sometimes also of butyl alcohol and acetone. Gram-positive.
The type species is *Clostridium acetobutylicum* Speakman.
5. *Peptoclostridium* Donker, 1926.
Rod-shaped bacteria, sometimes occurring in chains. Either immotile or motile by means of peritrichous flagella. Endospores formed, sometimes in typical clostridia or plectridia. Chemo-heterotrophic, anaerobic, capable of fermenting protein decomposition products with the formation of carbon dioxide, hydrogen and other unknown products. Gram-positive.
The type species is *Peptoclostridium putrificum* (Bienstock) Donker.

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LIFE'S FRINGES

FIFTY years ago, the first congress of our Society was opened by an exhilarating lecture of the then president, Stokvis. The first speaker next to address a plenary session was a microbiologist, Beijerinck, who in a splendid paper presented his ideas on a subject of microbial metabolism that is timely even to-day. After this event microbiologists have not, as far as I am aware, appeared before the footlights of our congresses, and consequently I deem it a particular privilege that I may terminate the first half-century of the existence of our Society with an attempt once again to illuminate to some extent a consuming problem in the field of microbiology. Apart from the fact that this science can only profit from an occasional confrontation of eminent proponents of the natural sciences with its problems, it is perhaps equally useful for another reason that a microbiologist every now and then may here raise his voice. This may be illustrated as follows.

Amongst the birds of different plumage who compose our membership, the biologists form a group that is more or less sharply differentiated from that comprising the students of the more exact fields of natural science. A careful observer cannot escape the conclusion that the biologist is characterized by a curious mixture of haughtiness and humility. The latter feature is the immediate consequence of the biologist's recognition that the results he obtains in often laborious toil lack the definitiveness that usually characterizes those of the mathematician, the astronomer, the physicist, or the chemist. But then, whence the superiority that evidently restores the mental equilibrium in the investigator of living nature? It, too, is understandable, for does not part of the glory of the object of a science reflect on him who earnestly and with devotion engages in its pursuit? And it certainly is not necessary to be a professional biologist in order to become impressed, at least now and then, with the astounding potentialities of living organisms. A moment of freedom from daily chores, a brief reflection on the accomplishments of man, animal, or plant, will suffice to render tangible the mystery of life. And then, how glaring is the contrast be-

tween the properties of living and those of inanimate matter! If, driven by the recognition of the mystery of life, the layman then takes cognizance of the results obtained by the investigators who aim at acquiring a comprehension of vital phenomena, one may reasonably anticipate that marvel will be added to admiration. For the number of manifestations of life on earth appears to be immense; the natural cycle of development traversed by all these living entities represents a well-nigh endless diversity. And whosoever rises above this impressive descriptive science to the heights of experimental biology, and considers, for example, the results of recent studies on the phenomena of regeneration, cannot fail to 'become deeply imbued with the impenetrable mystery of life, of the divine urge towards harmony that governs all living nature', as has been stated by Boeke. Truly, with such vistas before him, the biologist must be allowed his modicum of superiority; it is a proud feeling to be permitted to engage in the service that is the study of life.

Now the microbiologist, by which I mean only the scientist who studies the micro-organisms for their own sake rather than the investigator who has earned his epithet as biologist by the fact that he tries to protect the higher organisms, whether plants or animals, from microbial invasion, occupies a somewhat exceptional place among biologists on account of the peculiarities of the organisms he studies. Nevertheless, above all else he is apt to feel united with other biologists because he is imbued with the validity of Rubner's dictum: 'Was lebt, – ist Eins, daher muss trotz der Varianten, welche die einzelnen Spezies vorstellen, in ihrem Leben das gemeinsame Bild des Ganzen sich widerspiegeln'. How strikingly has not the truth of this passage been demonstrated, particularly during the past few decennia. Does one not, time and again, observe that the investigators of higher forms of life deign to descend to studies with one-celled organisms such as bacteria, yeasts, algae, etc., in order there to seek and frequently also to find the answers to their problems?

But this does not alter the fact that in many instances the mystery of life will force itself upon the microbiologist less directly. How often does he not sit bent over his microscope, torturing himself with the question whether some corpuscle in his field of vision may or may not lay claim to the glorious predicate 'living'? How simple, and in a sense transparent are not many of the chemical transformations per-

formed by microbes, so much so that present-day industry sometimes gladly makes use of them. Is it not pardonable if the microbiologist, who, by opening a single valve, daily causes *Clostridium acetobutylicum* rapidly to convert 50,000 gallons of corn meal mash into a solution rich in butanol and acetone, does not always keenly realize the mystery of life, and unconsciously considers the operation on a par with similar reactions in which, *e.g.*, strong acids are the causal agent?

This, indicated summarily, is why the microbiologist occupies an exceptional position, also in our association. Less haughty – probably less humble as well – than the student of higher forms of life, he seems predestined to form a natural link between the latter and the student of the more exact branches of natural science. I shall consider myself fortunate if the following discussion may contribute to an acceptance of this conviction in wider circles.

As already remarked, the microbiologist is as much imbued with the mysteriousness of life as any one else, during moments of introspection and reflection. This, for example, will happen when he observes with his microscope an actively moving, small bacterial type such as *Bacterium prodigiosum*, and delights for a while in the merry antics of the barely visible individuals. Here the mystery is again tangible, and inscrutable the sense of so minuscule an expression of life, the fate and destiny of every separate living individual. In these days, when the whale has claimed the attention of our entire nation, the microbiologist inevitably must ponder the overwhelming contrast in dimensions of such manifestations of life with those it is his lot to study; a contrast I can best illustrate by pointing out that, had I requested the draughtsman of the figure* to produce, by the side of the excellent likeness of *B. prodigiosum*, another one, representing the largest present-day animal, the whale, on the same scale, the figure should have a length corresponding to the earth's radius. Such thoughts lead the microbiologist to the question whether an organism like the above-mentioned bacterial species actually represents life in its smallest dimensions, and involuntarily he is tempted to drag out his microscope and search for increasingly smaller forms of life. And indeed he will book some successes, for bacteria such as *Spirillum parvum* and a few others, whose

* *Ed. note:* At the lecture figures of the dimensions of bacteria, viruses and large molecules were shown.

width amounts to only 300–400 m μ appear before his eyes. But then he will recognize that optical limitations prevent the direct observation of still smaller forms. Should one now conclude that here a most unexpected coincidence has come into play, and that the lowest limits of life concur with those that are specific to the optics of the microscope? This seems improbable, and inductively the microbiologist will reach the conclusion that there may well exist a realm in which the individuals are forever hidden from direct observation. It is generally known that numerous observations of various sorts have gradually accumulated which at first sight clearly force us to the same conclusion.

A single observation of this kind may here be briefly sketched. It might happen, and it actually has happened, that the same microbiologist who a while ago delighted in watching the antics of his tiny bacteria, would be induced to repeat his observations half an hour later, and in so doing, and to his utter astonishment, would find that the merry 'little animals' of a moment ago had completely disappeared from his slide; not even bacterial corpses interrupt the vacuity of the field. While our microbiologist tries to recover from the shock he has experienced, it might happen that the door to his laboratory is opened, and that his famous colleague, Félix d'Hérelle, enters. If then he communicates his experience, he would undoubtedly notice a pitying smile spreading over his visitor's face, and in all probability he would hear the answer: 'My dear colleague, don't you realize that your bacterium has fallen prey to the obligatorily parasitic ultramicrobe that I have discovered and named *Protobios bacteriophagus*?' And, with a view to reinforcing this verdict, the visitor might show that a minute fraction of the drop on the slide, added to a young, growing culture of the same bacterium, causes the latter with equal suddenness to clarify; that a tiny quantity of this culture effects the same in a subsequent culture; and so on and so forth, *ad infinitum*. He might also demonstrate that such a 'dissolved' culture can be passed through a filter that retains all particles of bacterial dimensions without thereby reducing its lytic power. He might, furthermore, show that it is frequently possible to dilute a lysed culture as much as 10⁹ fold with a previously sterilised solution, whilst preserving its lytic capacity. And he might provide the evidence that this power is destroyed by a brief heat treatment of the liquid, say at 70°C. In view of all this the conclusion

appears well-nigh unavoidable that the lysis of the bacteria is caused by a living organism of ultra-microscopic size that multiplies during the lytic process.

For that matter, the study of various diseases of man, animals, and plants had, already long before the discovery of the bacteriophage, yielded the conclusion that the causal agents of these diseases, too, were ultra-filterable living organisms. Also in these cases had the fact been demonstrated that the contagious agent can pass through filters that retain all microscopic micro-organisms; and it had been shown beyond a doubt that during its activity this agent multiplies in its host so that in this manner it may be propagated *ad infinitum*.

The concept of the existence of an invisible world in the strictest sense was strongly supported by the fact that shortly phenomena were established that unmistakably seemed to point to adaptations or to changes in the virulence of the types of virus studied. In short, these agents behaved in every respect in a manner that is equally characteristic of numerous parasitic microbes of microscopically observable size. Attention had, however, been drawn to the fact that all these virus types shared one common property, *viz.*, their inability to multiply in media devoid of normally observable living cells. But because this property had also been encountered among some few microscopically visible single-celled parasites, this observation did not necessarily disturb the confidence in the ultra-microscopic living world.

It is, however, self-evident that already at an early date the desire was felt to demonstrate more directly the corpuscular nature of the contagious agent present in a filtrate. A few years ago the British scientist, Barnard, succeeded in doing so with the aid of a highly perfected technique. He prepared photomicrographs with monochromatic ultraviolet irradiation and quartz optics. The consequent increase in resolving power made it possible to obtain images of certain virus types on the photographic plates. If one examines the reproductions of these images, *e.g.*, of the smallpox virus, the ectromelia virus of mice, and Kikuth's canary virus, and compares them with identically prepared photomicrographs of a small bacterial species such as *B. prodigiosum*, one fails to find grounds for protesting against a concept that considers these viruses as diminutive 'editions' of known microscopic parasites. Hence it might have appeared to many persons that the microbiologist had found his theoretically probable world of ultra-

microbes, and one might be inclined to conclude 'que tout était pour le mieux dans le monde des inframicrobes'.

Nevertheless it is understandable that numerous investigators cherished the desire to become more closely acquainted with the representatives of this invisible living world. Highly ingenious methods were developed for this purpose, and slowly but surely the knowledge of viruses of man, animal, plant, and bacterium increased. I shall deliberately neglect the historical development of this information, and right away mention one of the most recent results obtained in this field. The circumstances under which these most important observations were made are decidedly of great significance. It is but necessary to imagine the situation of a chemist connected with the Rockefeller Institute for Medical Research who decides to acquire a better understanding of the virus of a plant disease by means of the sober and clear-cut methods of modern biochemistry. As experimental material he chooses the causal agent of the tobacco mosaic disease, the classical affliction that Iwanowski in 1892 first ascribed to a filtrable, hence submicroscopic living agent, and which six years later led Beijerinck to his bold hypothesis of a '*contagium vivum fluidum*'.

It was already known to Iwanowski that a tobacco plant suffering from the mosaic disease can yield a press juice which, even after filtration through filters of a normal pore size, is highly infectious for healthy plants. Here, therefore, we have a clear liquid that contains, besides a large number of soluble plant constituents, the causative agent of the disease. To a biochemist with up-to-date training, W. M. Stanley, the notion was obvious that one might attempt to separate the agent from the other substances by fractional precipitation and selective adsorption, checking the distribution of the causal agent in the various fractions at each step by infection experiments on a quantitative basis. In order to accomplish the latter he used a method first devised by Holmes in 1929. It is based on the fact that in leaves of *Nicotiana glutinosa*, in contrast to what happens in *N. tabacum*, the virus causes strictly localized damage. Thus it suffices to smear half of a leaf with a virus-containing liquid, using the other half as a control; this permits a straight-forward count of the number of virus particles present in the solution. By extended trials Stanley succeeded in eliminating more and more inactive material, finally winding up with a

clear solution that contained the bulk of the disease-producing capacity. Addition of ammonium sulphate to this solution caused the formation of a precipitate, leaving the supernatant liquid practically devoid of virus. Conversely, 1 ml of a solution that contains only 10^{-9} g of the precipitate can cause a healthy plant to become diseased. The precipitate is soluble in water, and can be reprecipitated by the renewed addition of ammonium sulphate. Even after a ten-fold repetition of this procedure the disease-provoking capacity of the final precipitate has not appreciably diminished in a quantitative sense. Who would hesitate to conclude that the salt addition had caused the ultramicroscopic virus to flocculate out as an amorphous and macroscopically visible mass? But Stanley examines a fraction of this mass under his microscope and observes . . . crystals!

This may make you realize the dramatic conflict that during the past few years, even to some extent prior to Stanley's discovery, has ensued with respect to the once so readily accepted world of the invisible life. Does this life really exist, or is that which at first sight suggests itself as such merely dead matter exhibiting some of the most characteristic features of life? Here one must consider particularly the capacity to multiply in an appropriate heterogeneous environment. This should not be minimized; and one must realize the portent of the following statement: a few of Stanley's tiny crystals, inoculated into a healthy plant, make it possible to obtain from this plant, after a month or so, 2 g of completely identical crystals per kg of press juice, while uninoculated control plants are entirely devoid of this material at the end of this period.

Is it, in view of the foregoing, surprising that in microbiological circles a passionate fight has been engendered, whose ultimate outcome must determine the question 'living or dead' for each virus separately? One must fully realize the tragic consequence of this state of affairs. Life proud and dignified, instantly recognized in the case of man and animal, of oak and rose, life that even the child senses as a special quality, and that the mature mind has learned to consider as an enclave in the continuum of inanimate matter whose limits can be surpassed only from inside to outside, this life, in its most minute manifestations, appears to be indistinguishable from some paltry crystals! No clear line of demarcation can be drawn in the realm of ultramicroscopic structures between the living and the non-living. Whoever

tries to distinguish life in these regions does not observe sharp contrasts and contours; there appears to be nothing but misty fringes . . . This situation may justify the title I have chosen for my lecture.

It will be evident that the above account was meant to sketch out the problem and to indicate its portent at least in rough outline. If I should have had to limit myself to this I should not have ventured to discuss this subject at the end of a congress during which so many impressive advances in the field of natural science have once again been reported. Even though I shall not succeed in dissipating the mist in which life appears to lose itself, there have been numerous investigators who have managed to penetrate further, and in the yet dim glow of the lights they have kindled a few vague outlines are nonetheless beginning to take shape. Some of these developments I should now like to communicate.

In the first place the question arises whether Stanley's crystalline tobacco mosaic virus implies the death-blow of submicroscopic life in general. It is understandable that nowadays the immediate reaction is prone to be a denial of the necessity to consider all filtrable infectious agents right away from a single viewpoint. This implies therefore that one certainly wishes to retain the belief in the existence of living entities, essentially similar to bacteria, though distinguishable from the latter because by their size they evade direct microscopic examination. This view is supported by the fact that recently Laidlaw and Elford isolated from sewage a microbe that exists on the one hand in the form of structures with a diameter not exceeding $125-175\text{ m}\mu$, as shown by strictly dependable filtration procedures, and on the other hand gives rise to larger bodies, directly observable by microscopy. Its most interesting aspect is, however, that it is a typical saprophyte, thus emphasizing the similarity with the majority of the smallest visible organisms. We may therefore conclude that submicroscopic life actually exists; and yet, at the other extreme, we find Stanley's crystals.

How may we expect to answer for each of the hundreds of viruses described thus far the question, inframicrobe or not? Obviously we may assume that an accurate determination of particle size will help out in this connexion. As early as 1906 Errera has pointed out that a spherical particle of $100\text{ m}\mu$ in diameter can contain only 10,000 protein molecules, a number that Errera considered as approximating the

minimum requirement for something that could lay claim to being designated as a living cell. For it must be realized that a further decrease in diameter causes a rapid decrease in the number of protein molecules that can be accommodated in the particle.

Owing to numerous excellent investigations the size of various proteins and of many different viruses has lately been determined with a fair degree of precision. With reference to proteins we owe this primarily to the brilliant work of Svedberg and collaborators with the ultracentrifuge they designed, and in which forces of up to a million times gravity cause a sedimentation of dissolved protein molecules. The sizes of virus particles have been determined by Bechhold, and particularly by Elford and co-workers, who succeeded in imparting a hitherto unachievable degree of reliability to filtration procedures by preparing series of collodion filters with decreasing and very uniform pore sizes, the so-called gradocol filters. With a relatively simple centrifugation apparatus Elford, during the past year, has moreover managed to obtain results that are in very satisfactory agreement with those of the earlier ultrafiltration method.

Some of the most important data have been summarized in the figure.*

It shows first of all a striking continuity; from the diameter of the smallest microscopically visible organisms to that of some of the most common proteins we encounter all possible transition stages. Among the viruses we find some whose size does not differ much from that of microscopically observable bacteria, and, at the other extreme, those whose diameter amounts to only 10–12 μ , as, for example, the foot-and-mouth disease virus, or that of poliomyelitis. The fact is worthy of attention that a particular protein, *viz.*, hemocyanin, the respiratory pigment of the snail, *Helix*, has a diameter twice that of these viruses, so that its volume is greater by a factor of 8. It is remarkable that the bacteriophages so far investigated also differ considerably in size.

The principal point is, however, that size cannot be used as a basis for sharply differentiating between ultramicrobes and other viruses; Errera's line of demarcation at 100 μ separates viruses that in many respects show the most pronounced similarity. Conversely, it is not surprising that the very small dimensions of many viruses have markedly contributed to the attitude of numerous investigators who, al-

* See note page 331.

ready long before Stanley's discovery, had denied these viruses the quality of autonomous parasitic living organisms.

But what are the consequences of this recognition? If one realizes that viruses can be characterized only by virtue of the destructive effects they exert on particular cells, and remembers that this effect is primarily characterized by a multiplication of the destructive agent itself, the denial of autonomous existence to this agent almost inevitably implies that it must be derived from the host cell itself. In consequence many investigators are inclined to regard the virus – and this applies particularly to the bacteriophage as well – as something produced by the infested cell which has been designated as enzyme, metabolic disturber, lethal factor, hereditary factor, or, simply, gene.

It will immediately be sensed that even such a concept entails many difficulties. The unlimited propagation at the expense of the living cell, the consequence that these cells must always carry the agent, or, at least, the potentiality to produce the agent of the specific disease by which they are ruined, these make it hard to reconcile this concept with the general experiences concerning the diseases involved. But it will be evident that the basic principle common to all these ideas would triumph if one were to succeed in unleashing the destructive agent by a non-specific intervention in the life of the cell, and, furthermore, in demonstrating the unlimited transmissibility of this agent.

Some very remarkable results have been obtained along these lines. It is the great merit of the eminent virologist, Doerr, to have shown long ago that it is possible by utterly different non-specific treatments to induce at will in perfectly healthy humans a typical herpes rash, and, as a corollary, to have proved that in the thus caused skin eruptions there is found a submicroscopic, filtrable agent with which the disease can be perpetually transmitted to man and experimental animals. A fully comparable situation has now been demonstrated to exist beyond a doubt in the case of various malignant tumors. These can be elicited in healthy animals by injecting them with tar or arsenite, and they can thereafter be transmitted at will by tissue filtrates. Moreover, the literature contains several accounts of cases in which the experimenter has succeeded in evoking bacteriophage-like phenomena in entirely normal bacterial cultures by means of certain non-specific procedures. In this connexion the recent observations of Kendall are of importance; he could induce phage formation in three different

bacterial species by the addition of previously autoclaved sewage to the culture medium.

Nevertheless, all these experimental results have not convinced the adherents to the theory of parasitic ultramicrobes. They defend the rather improbable view that in all these cases the ultramicrobe already exists in a latent form in the healthy experimental animal or in the normal bacterial culture. The corresponding non-specific procedure would simply reduce the resistance of the host against infection, and thus enable the parasite to multiply.

For a while it seemed as if it would be possible to unnerve this counter-argument. This was when our fellow-countryman, Den Dooren de Jong, managed to liberate bacteriophage from cultures of a sporeforming bacterium that had been started with bacterial spores previously heated at 100 °C. Since it has been the common experience that the most divergent bacteriophages are inactivated by a brief exposure to a temperature of 75 °C, this presumably provided the ideal situation in which a living organism, previously and with certainty rendered free of phage, even of latent phage, could nonetheless be induced to produce the virus as a result of the application of certain measures. Unfortunately this experiment, too, signified no more than a local break-through in the trench warfare between the adherents and the opponents to the theory of the autonomous parasite. The proponents invented the rather unlikely 'theory of the safety-deposit box', according to which the bacterium is kind enough to permit the ultramicrobe-parasite to share the benefit of the thermoresistent properties of the bacterial spore, and it must be admitted that a number of experimental data do indeed favour this view, at least at first sight. Thus the equilibrium between the two opposing forces has again been restored.

I do not wish to tire out my audience by discussing in detail the various other arguments that have been brought to bear on the problem by both contending parties, and the attempts that have been made to refute them. Rather shall I try to circumscribe the present position as follows. In favour of the theory of the autonomous ultramicrobe must be considered the great similarity in behaviour of filtrable agents and of microscopically discernable single-celled parasitic organisms; good examples of this are found in the adaptive phenomena, changes in virulence, and the immunological properties. On the opposite side

one finds the numerous arguments that render it inadmissible to regard the said agents as autonomous living entities, and of which I mention only the extremely small dimensions, the specific gravity – which, being about 1.25, lies much closer to that of a protein (1.35) than of small bacterial cells (1.10) – the high resistance against typical and universal cell poisons such as mercuric chloride, and the prolonged preservation of these entities under conditions where multiplication is virtually excluded; frequently viruses have been found to retain their properties in a quantitatively undiminished degree even after storage for periods of up to ten years. Furthermore, we must remember the strong indications in favour of the non-specific genesis from normal living cells which is also supported by the fact that some infectious diseases must almost certainly be characterized as originating spontaneously. And finally the strongest argument of all, *viz.*, Stanley's proof that a virus can be isolated in crystalline form. In this manner we can visualize the impasse of the ultramicrobiologists.

Even though the way out cannot be sharply outlined, an attempt may nevertheless be made to indicate in what direction it will have to be sought. To me personally it is beyond a doubt that it will be biochemistry that is apt to bring us nearer to a solution. Starting with the viruses which, like the tobacco mosaic virus, can be obtained in chemically pure form, this branch of science will permit us to characterize more adequately the chemical nature and potentialities of those extremely small particles that can exert such impressive and often devastating effects. But primarily biochemistry must help us to acquire an understanding of the manner in which the penetration of the virus into a metabolizing cell causes the latter to shift its normal synthetic functions in the direction of synthesizing a virus as a by-product.

Concerning the first point of this programme it can be stated that the initial steps have already been taken, principally by Stanley and collaborators. It is obvious that for this kind of work everything depends on the certainty that the isolated crystals actually represent the virus proper. I have already mentioned Stanley's primary proof for this contention, *viz.*, the repeated recrystallization, now successfully accomplished fifteen times, with full retention of activity. Nevertheless, I am convinced that there are many among my audience who are not prepared to accept without more ado an identification on this basis. Permit me therefore to mention some further results obtained by

Stanley that similarly favour this view. In the first place it has been unambiguously shown that numerous treatments, in part very mild ones, that deprive a solution of the crystals of its activity as a virus, also cause more or less radical changes in the protein. Separation of protein and virus by ultrafiltration appeared impossible; if the pore size of the filter was small enough to retain the protein, the filtrate was invariably inactive.

Particularly convincing are the results published in a recent communication showing that during ultracentrifugation of solutions of the crystalline protein the infectivity disappears from the upper layers at the same rate as that at which the protein is removed as a consequence of the sedimentation.

The study of the 'aucuba' variety of the mosaic virus has also yielded a crystalline protein that differs merely in some minor details from the typical mosaic virus. Inoculation of tomato plants with the tobacco mosaic virus also produced a crystalline protein that appeared to be identical in every respect with the protein isolated from diseased tobacco plants. An important additional fact is, furthermore, the demonstration by Bawden and co-workers that the juice from healthy tobacco plants does not contain this protein. Moreover, Stanley and Wyckoff have recently reported that by means of alternating centrifugation and ultracentrifugation they have succeeded in isolating, again in crystalline form, the very unstable causal agent of a third virus disease of tobacco, the so-called ringspot disease. This, too, appeared to be a protein; it differed markedly from the mosaic virus in its chemical, physical, and serological properties, as could have been expected.

In view of this assembly of facts the identity of viruses and the isolated proteins appears irrefutable. It is therefore important to find out what has become known concerning the chemical nature of these proteins. The X-ray diagrams of the mosaic and aucuba virus, determined by Wyckoff and Corey, agree in essence completely with those of earlier investigated proteins. Sedimentation analysis with the aid of the ultracentrifuge showed convincingly that the molecular weight of the virus protein is very large, and exceeds 10,000,000. Svedberg, in a later investigation, obtained a value of 17,000,000. The significance of this fact becomes clear if viewed in conjunction with the fact that the juice of healthy tobacco plants does not contain any proteins with a molecular weight over 30,000 in appreciable amount. In dilute

solutions of the virus protein the large molecules seem to disintegrate with the formation of smaller units that are more resistant to sedimentation.

A highly important point is, moreover, that most preparations evidently contain small amounts of nucleic acids, although these can be eliminated by dialysis under special conditions without thereby causing any loss in virus activity.

Apart from the causal agents of the above-mentioned plant virus diseases yet another virus has been obtained in a state of sufficient concentration and purity to permit some account concerning its composition. This is the coli bacteriophage which Schlesinger isolated in virtually pure, though not crystalline state. Also this preparation appeared to be a protein. The most characteristic analytical result was the high phosphorus content (3.7 per cent); this, together with the later reported positive outcome of the Feulgen test, leads to the conclusion that the bacteriophage is, at least predominantly, composed of nucleoprotein. The virus of foot-and-mouth disease, far more difficult to isolate in large amounts, has been shown by Janssen to be a phosphorus-containing protein as well. Noteworthy is furthermore that Schüler could ascertain with a very concentrated and active bacteriophage preparation that this material does not by itself exhibit any metabolic activity; in media with organic substrates but devoid of bacteria it causes neither consumption of oxygen, nor production of carbonic or other acids.

In summary we may therefore conclude that observations in various directions converge in the concept that at least the smaller viruses are high-molecular proteins, attached more or less firmly to nucleotides or nucleic acids.

It is now possible to formulate somewhat more clearly the problem confronting the biochemists in relation to these results in the following manner: what factors determine the synthesis of this group of compounds in the host cells? This is a question that at first sight seems so stupendous that one might well despair of ever finding an answer to it. Nevertheless, here, too, one can discern a spot of light; because a more careful consideration makes it clear that the synthesis of a virus protein is not an isolated problem; ultimately it belongs to the same category as the problem of biochemical synthesis in general.

Now it is a most remarkable fact that virtually all biologists consider

the phenomenon of growth, of cell multiplication, as something that is inherent in life and can be studied only as far as its dependence on external factors is concerned. Rarely does one encounter signs of a recognition of its implications in a chemical sense. And yet it implies no less than that the living cell manufactures, often from one single organic nutrient substance, the hundreds of compounds, some of them extremely complicated, that one finds when analyzing the chemical composition of cell material.

It seems to me that this concept has so far been grossly neglected, also by biochemists. Violent combats have been fought by these scientists over their attempts to comprehend how a yeast cell manages the relatively simple conversion of a glucose molecule into alcohol and carbon dioxide. But how many among our biochemists have ever troubled themselves about the problem how that same yeast cell succeeds in converting other sugar molecules into the fats, lipoids, steroids, hemins, flavins, amino acids, proteins, nucleoproteins, and so on and so forth, that nonetheless must be produced with equal certainty?

To be sure, it is, as usually, much easier to raise the question than to answer it. But viewed from the standpoint of the virus investigations there is much comfort in the consideration that the miracle of the new-formation of virus in the host cell is no more than a special accessory to the every-day phenomenon of biosynthesis. It will be necessary clearly to keep in mind the fact that every cell synthesizes nucleoproteins from simple building blocks; and in that case the virus problem can be reduced to the rather more simple question: 'Why is the composition of this nucleoprotein occasionally modified?' May we not hope that, once the mechanism of the normal biosynthesis has been more clearly defined, the solution of the other problem will not cause too many difficulties?

As a biochemist I am pleased to state that the earlier expressed deficiency in biochemical approaches is not general, and that there exists at any rate one organisation where, for a considerable period of time, the viewpoint here defended has been heeded. And as a Netherlander it gives me satisfaction to say that this organisation is a Dutch one. Although generally the Netherlands' commonwealth seems to hold the opinion that the purposeful promotion of experimental science is no concern of the state, and appears to believe that in our country pure science can be satisfactorily developed as a by-product

of higher education and of economically valuable direct applications of scientific research, there are, however, external factors, largely international pressure coupled with powerful export interests, that have incidentally forced the Netherlands' government to make an exception for the field of virus research. In the State Veterinary Research Institute in Rotterdam a number of scientists have, for several years, been in a position to devote themselves entirely to the study of the virus of foot-and-mouth disease. Under the active guidance of the Director, Dr. H. S. Frenkel, several important results have already been obtained, especially pertaining to the *in vitro* cultivation of the virus in tissue cultures. In conjunction with the present problem I must refrain from discussing these advances; but I wish to emphasize that the Director immediately realized that the planned investigations could not be expected to bear fruit except through the co-operation of a biochemist. Now this biochemist, Dr. L. W. Janssen, in addition to carrying out his successful experimental studies, *e.g.*, on the purification of the virus, has become increasingly engrossed in the general problem of biosynthesis. In consequence he has developed some ideas that unquestionably merit our full attention, and with Janssen's kind permission I am in the fortunate position of being able to communicate some of his as yet unpublished results. I need not mention that I can only give you a glimpse of these fruits of prolonged cogitation and extensive studies of the literature; a final evaluation of Janssen's contribution should therefore be postponed until his publication, now in preparation, has appeared in print. Briefly summarized, his results imply that he has demonstrated the feasibility of interpreting the genesis of the many hundreds of very heterogeneous organic compounds, encountered as multifarious mixtures in man and in the most divergent representatives of the plant- and animal kingdoms, as the end result of transformations of groups of glucose molecules in such a manner that the initial and final stages are separated by a small number of oxido-reduction and condensation reactions that proceed with astonishing regularity. An important aspect of the proposition is that the gradual transformation of the sugar molecules is conceived as progressing on an 'assembly belt' which has a structure composed of nucleoproteins with the arginine components of the proteins as well as the nucleotide moiety representing the essential agents for the transformations. I cannot discuss in detail the many merits of the ideas advanced

by Janssen; these are, moreover, in striking agreement with what has lately been learned concerning the structure of proteins. I may, however, present a bird's eye view of a single one of the hundreds of schemes constructed, in which I must call particular attention to the concept concerning the synthesis of the simplest nucleoprotein; the principle can be extended at will to the more complex members of the group.

Eminent scientists have recently introduced the derogatory term 'paper chemistry' to indicate attempts such as Janssen's. I, too, fully realize the conjectural nature of these ideas based on deduction; but I feel compelled to stress two points in this connexion. Firstly, Janssen's working hypothesis covers a phase of biochemistry that at present can best be characterized as a vacuum. Secondly, it is tempting to coin, as a counterpart for 'paper chemistry', the term 'paper physics', and this immediately suggests accomplishments of leading scientists which, in my opinion, clearly emphasize the right of biochemists, too, to formulate, prior to their experimental work, hypotheses that can serve as guides for their investigations.

This may suffice; in the framework of the virus problem it is enough to realize that out of the mist that surrounds us a vague possibility emerges to the effect that some day a well-founded concept will develop concerning the synthesis by a living cell of a nucleoprotein from non-specific building blocks for which a nucleoprotein constituent of the cell is directly responsible, so that the nature of the newly formed molecule will be determined by the configuration of its progenitor, and may even be identical with it.

Now this viewpoint assumes a most suggestive aspect if it be considered in relation to the recent experimental results of another phase of biochemistry, *viz.*, the respiratory mechanism, which is gradually being elucidated. The masterly investigations of Warburg and collaborators have, in fact, unequivocally proved that the simple dehydrogenation of an oxidizable substrate by a yeast cell is also mediated by a nucleoprotein. This primary agent is formed as a loose compound if a specific protein is brought in contact with a nucleotide whose chemical composition as nicotinamide-adenine-ribose-phosphate has meanwhile been elucidated almost completely. Remembering Stanley's crystalline viruses, is it not extremely remarkable that quite recently Negelein has reported that he has isolated also the protein carrier of the substrate dehydrogenase in a crystalline form by precipitation

with ammonium sulphate? In agreement with its modest function, the molecular weight of this biocatalyst is probably not more than 70,000; a low value that sharply contrasts with that of the giant virus molecules to which must, after all, be ascribed the much more radical rôle of a catalyst for synthetic oxido-reductions.

But the recent studies of Warburg and co-workers are of particular importance in connexion with the following. According to a preliminary report, Warburg and Christian have found that the above mentioned nucleotide can form complexes with various proteins isolated from yeast, each complex acting as a dehydrogenation catalyst. Now, whereas one complex dehydrogenates an oxidizable substrate like Robison ester to the corresponding phosphohexonic acid, another performs a much more extensive oxidation which even leads to the liberation of carbon dioxide. Here we see, therefore, an oxido-reduction reaction chain unmistakably determined by the nature of the colloidal protein carrier.

If now we return into the mist of the virus field we may try whether, by the light of this extremely interesting result, we can begin to distinguish some shapes also here. Is it not very tempting to imagine, in connexion with Janssen's line of thought, that the virus protein after its penetration into the cell takes possession of the nucleotides or nucleic acids that function as prosthetic groups in the normal cellular syntheses? The result would be a shunt of the normal biosynthesis in the direction of a synthesis of virus protein. That is to say that along with a disruption of the harmony of the various synthetic entities of the infected cell there will occur a multiplication of the virus. Is not such a concept eminently suitable to render understandable also the alarming increase in the number of plant virus diseases that has been noted by all phytopathologists? Might not this be the result of the fact that the much more intensive cultivation of recent years has greatly increased the chances for contact between various plants, thus opening the possibility that synthetic entities of certain species might find their way into the cells of different species? May this not also throw light on the still so mysterious behaviour of 'virus carriers', apparently healthy varieties of cultivated plants that nevertheless can transfer virus diseases to sensitive clones? Cannot one accept more readily the conclusion that a particular normal synthetic entity of the carrier race causes the disorganisation of the cells of a sensitive variety than the as-

sumption that the carrier race, without exhibiting any disease symptoms whatsoever, harbours in all its parts a species-foreign virus protein?

And the earlier discussed non-specific virus induction that has been made so highly probable obviously fits completely into this same train of thought. Is it not conceivable, for example, that the non-specific agents that cause Doerr's experimentally induced herpes destroy particular synthetic entities, and that the consequent disruption of the harmony of the cell leads the remaining synthetic entities, perhaps modified by the interference, to a directed synthesis of the protein that acts as the herpes virus? Must we not then also regard the filtrable, transmissible, tar-induced sarcomas in the same manner, with the difference that in this case the production of the virus is accompanied by a new equilibrium between the remaining synthetic entities, an equilibrium that we designate as a sarcoma?

But are we not thus led to consider these synthetic entities more generally as the 'constitutive vital elements' of the living cell? Involuntarily one is here reminded of a statement that was made, though in my opinion quite inopportune, by d'Hérelle:

'Toute la biologie reposait, repose encore sur l'hypothèse fondamentale que l'unité de la matière vivante c'est la cellule. Beijerinck le premier s'est affranchi de ce dogme et a proclamé le fait, que la vie n'est pas le résultat d'une organisation cellulaire, mais dérivée d'une autre phénomène qui ne peut dès lors résider que dans la constitution physico-chimique d'une micelle protéique.'

Would not this also indicate how we may solve the difficulty inherent in the notion that at some future time a sharp boundary shall be drawn between the filtrable contagia that may lay claim to the glorious epithet 'living' and the inanimate crystalline virus proteins? We may, I believe, expect that, like the Versailles treaty, a peace treaty based on this concept will remain a constant source of disquiet and friction. In view of the startling analogy in the activity of the most divergent viruses, the adherents to the theory of autonomous parasites would evidently find it an intolerable notion that the proud vital activities are so perfectly imitated on the other side of the dividing line.

Must we not search for a genuine pacification by way of the concept that the cellular organisation of life has been preceded by an evolution that has started with still more elementary living units and

tended to the appearance of a living cell? In essence this would not be different from *the* evolution of the biologists which leads from single-celled organisms to the most highly organized manifestation of life. And could not then the various submicroscopic expressions of life represent different stages in this pre-cellular evolution? At the same time, this would imply the existence of a continuous transition from the simple nucleoprotein to the smallest unequivocally organized cell.

Mr. President, ladies, and gentlemen; the title of my discourse should have been a warning to you. You have not been deterred by it, and you have accompanied me in this exploration of life's fringes. Where a clear view is wanting it is easy to get lost in meditations; but after returning home the memories are quickly superseded by impressions that are more distinct and provide greater satisfaction.

At the finish I should like to make another remark. Toward the end I may for a moment have evoked the notion that after many years, when the mists shall have become rarefied, the concept may have found acceptance that there is a continuity from inanimate to living matter. But please do not be misled into believing that I have lost sight of the fact that nowadays prominent biologists believe that the essence of life should be sought in the very pattern of its proclivities which cannot be explained on the basis of a mere causality. But it is possible, I believe, that a closer investigation of submicroscopic life will reveal similar tendencies in a more elementary form also in this realm, just as these are known in modern atomic physics. In consequence we might paraphrase the adage, 'Go to the ants, thou sluggard' by the admonition, 'Study the virus, thou theoretical biologist!'

And finally: in all of us lives a profound awareness of the unfathomableness of life's enigma. Nevertheless, it seems justifiable to raise the question whether this is irreconcilable with the concept of a spontaneous transition from inanimate matter to life, and a correspondingly unifying evolution to its higher forms. Does it mean that we minimize the miracle of life if we let it merge with the miracle of creation itself? Or, conversely, might not the miracle of life's origin be thus freed from the limitation which part of humanity attaches to it, *viz.*, the limitation of its belonging to the past, rather than to the present, the future, eternity?

DIE KOHLENSÄURE IM STOFFWECHSEL DER LEBEWESEN

BEVOR ich zu meinem Thema übergehe, ist es mir ein aufrichtiges Bedürfnis Ihnen zu versichern, dass ich die ehrenvolle Einladung des Professorenkollegiums der Finnischen Technischen Hochschule hier für Sie vorzutragen sehr hoch schätze. Und dies um so mehr weil diese Einladung mir die Möglichkeit eröffnet hat durch eigene Anschauung mit dem so renommierten Biochemischen Institute bekannt zu werden in dem das Mitglied Ihres Kollegiums Professor Virtanen und seine Mitarbeiter solche wichtige Entdeckungen auf dem Gebiete der Mikroben- und Pflanzenphysiologie gemacht haben.

Das Thema, welches ich heute mit Ihnen besprechen möchte, bezieht sich auf die Rolle der Kohlensäure im Stoffwechsel der Lebewesen.

Auch diejenigen, welche keine professionellen Physiologen oder Biochemiker sind, pflegen vertraut zu sein mit der grundlegenden Bedeutung der Kohlensäure im Kreislaufe des Kohlenstoffs auf der Erde. Ein jeder, der auch nur über oberflächliche naturwissenschaftliche Kenntnisse verfügt, ist davon durchdrungen, dass diese in chemischer Hinsicht so einfache Verbindung gewissermassen einen Knotenpunkt der beiden beim ersten Anblick im Kreislauf zu unterscheidenden Hälften bildet. Einerseits ist ja die Kohlensäure die Muttersubstanz für die Synthese der organischen Materie, eine Synthese, welche unter Mitwirkung der Sonnenenergie in den mit Chlorophyll ausgerüsteten Pflanzenzellen stattfindet. Andererseits werden die meisten unter Ihnen sich bewusst sein, dass diese Synthese sich auf der Erde nur deshalb fortwährend vollziehen kann, weil die dabei gebildete organische Substanz unter dem Einflusse der nicht-chlorophyll-führenden Organismen – tierische sowie auch pflanzliche (Pilze und Bakterien) – wiederum in Kohlensäure zurückgeführt wird.

Man deutet diesen letzten Vorgang öfters als ‘Mineralisationsprozess’, mit welchem Namen man zum Ausdruck bringen will, dass die Welt der farblosen Organismen den Kohlenstoff der organischen

Substanz in die einzige Kohlenstoffverbindung überführt, welche der anorganischen Chemie (französisch: 'chimie minérale') angehört. Aus dem anorganischen Reiche wird dann der Kohlenstoff wieder zur organischen Substanz, d.h. zum Träger des Lebens erhoben, dank dem vorläufig unerschöpflichen Energiestrome, welchen die Sonne durch ihre Strahlung auf die Erde ergießt.

Dies ist allerdings das einfache Bild, welches wir alle aus den ersten Jahren unseres Biologie-Unterrichtes kennen. Wer von uns ist nicht getroffen von der klaren Logik, welche aus diesem biologischen Weltbilde hervortritt? Wer von uns hat aber daraus auch nicht einen starken Eindruck bekommen von der ganz einzigartigen Stellung, welche die Kohlensäure inmitten der übrigen Kohlenstoffverbindungen einnimmt? Können doch alle diese Verbindungen biochemischen Verwandlungen, öfters sehr verschiedener Natur, unterliegen. Demgegenüber steht die Kohlensäure als unangreifbares Endprodukt des Stoffwechsels der farblosen Organismen, sich gleichsam ansammelnd in einem 'Tal des Todes', woraus sie nur mit Hilfe der Lichtenergie wieder erhoben werden kann.

Es fragt sich nun jedoch, in wieweit dieses so befriedigende primäre Bild sich auch bei dem Fortschritte der Wissenschaft behaupten lässt, oder ob es nicht in Folge neuer Beobachtungen mehr oder weniger retuschiert werden müsste.

Sogar eine oberflächliche Überlegung genügt zur Einsicht, dass die bis jetzt gegebene Skizze doch wohl etwas zu schematisch gehalten ist. Denn eine nähere Betrachtung des Kreislaufes des Kohlenstoffes lehrt dass eine Umwandlung der organischen Substanz in Kohlensäure unter dem Einflusse der farblosen Organismen sich niemals sofort in quantitativer Weise vollzieht. Auch diesen Organismen fehlt das synthetisierende Vermögen keineswegs: ein Teil der Nahrung wird immer in Leibessubstanz übergeführt.

Inzwischen wird auch dieses synthetische Material nach dem Tode der Organismen in einen neuen Zyklus einbegriffen werden, wobei aufs neue ein ansehnlicher Teil des darin vorhandenen Kohlenstoffes in Kohlensäure übergeführt wird. Der dieser Überführung entwischte Teil wird jedoch seinerzeit wieder in einen neuen Zyklus einbezogen werden, und diese Vorgänge wiederholen sich viele Male, bis endlich die Umlagerung der organischen Substanz in Kohlensäure doch praktisch vollständig geworden ist.

Obleich die geschilderte Komplikation eine Verzögerung im Schliessen des Kohlenstoffkreislaufes mit sich bringt, wird doch das primäre Bild dadurch nicht prinzipiell geändert.

Aber auch der synthetische Teil des Kreislaufes erweist sich bei tiefer gehender Analyse weniger einfach als man beim ersten Anblick meinen könnte. Bis jetzt habe ich, wie üblich, 'die grüne Pflanze' als eine andere, höhere Kategorie den chlorophyllosen Organismen gegenübergestellt. Aber auch diese Ansicht lässt sich bei näherer Überlegung nicht behaupten. Seit langem ist ja bekannt, dass auch die chlorophyllführende Zelle in Abwesenheit von Licht in biochemischer Hinsicht sich den farblosen Zellen völlig analog verhält. Auch die grünen Zellen werden doch unter bestimmten Bedingungen ebenfalls nicht unerheblich zu einer Verbrennung der organischen Substanz, d.h. zu der Produktion von Kohlensäure, beitragen.

Hierzu gesellt sich dann noch eine zweite, zu oft vernachlässigte Überlegung, welche ebenfalls die Sonderstellung der 'grünen Pflanze' erheblich schwächt. Sogar eine oberflächliche anatomische Analyse der grünen Pflanze lehrt nämlich, dass die Anzahl der chlorophyllführenden Zellen nur einen meist bescheidenen Bruchteil der Anzahl der im ganzen im grünen Organismus vorhandenen Zellen bildet. Zu selten macht man sich klar, dass der grössere Teil der grünen Pflanze aus Zellen aufgebaut ist, deren Stoffwechsel mit demjenigen der tierischen und farblosen pflanzlichen Organismen völlig übereinstimmt. Auch aus diesem Grunde trägt die grüne Pflanze auf nicht zu unterschätzende Weise zu der Mineralisation bei.

Es ist deutlich, dass durch diese Betrachtungen der Kontrast im primären Bilde erheblich geschwächt wird. Dem steht jedoch gegenüber, dass dadurch auch ein Vorteil erreicht wird, indem die Einheit in die Welt des Lebendigen weitgehend wiederhergestellt wird. Einerseits fehlt den farblosen Organismen das synthetisierende Vermögen keineswegs, andererseits ist die grüne Pflanze auch an dem Abbau der organischen Substanz wesentlich beteiligt. Die grüne Pflanze erweist sich bei diesen Betrachtungen als ein Organismus wie jeder andere, nur hat sie die Eigenart, dass sie über einen unter bestimmten Verhältnissen wirksamen photosynthetischen Apparat verfügt.

Die bis jetzt gegebenen Betrachtungen ändern jedoch nur unsere Ansichten über die Natur der bei der Synthese und bei dem Abbau wirksamen Agenzien. Die Stellung der Kohlensäure wird davon gar

nicht angegriffen. Sie bleibt das fast 'heilige' Molekül, welches sich im Gedränge der Stoffwechselprozesse hoch und hehr in den Zellen bewegt. Nur da, wo dieses Molekül mit von der Lichtenergie aktivierten Chlorophyllmolekülen in Kontakt kommt, unterliegt es der Gefahr seine chemische Individualität zu verlieren. Jedenfalls hat es während langer Zeit diesen Anschein gehabt.

Allmählich hat aber auch diese Lage sich geändert. Die Fragen, in welchem Masse dies geschehen ist und wohin die eingetretene Evolution der Ansichten uns jetzt schon geführt hat und uns wahrscheinlich in der Zukunft noch führen wird, bilden den Gegenstand der jetzt zu gebenden Ausführungen.

Es wird vielen unter Ihnen bekannt sein, dass der erste Eingriff in die Sonderstellung der Kohlensäure zurückzuführen ist auf die Entdeckung der Existenz der sogenannten chemoautotrophen Organismen. Die Geschichte dieser Entdeckung ist sehr interessant und ist in den Lehrbüchern oft verzerrt wiedergegeben. Ich möchte sie daher hier kurz skizzieren.

Im J. 1886 veröffentlichte der deutsche Bakteriologe Heraeus [1886] ein Studium über die im Boden weit verbreiteten Bakterien, welche instande sind Ammoniak zu oxydieren. In dieser Abhandlung sprach der Autor die Vermutung aus, dass diese Bakterien das Vermögen besäßen, im Dunkeln ihr Zellmaterial aus Kohlensäure zu synthetisieren. Im J. 1887 wird diese Annahme von seinem Lehrer Hueppe [1887] auf Grund qualitativer Beobachtungen bestätigt. Dass Hueppe sich von der umstürzenden Natur dieser Annahme wohl bewusst war, geht daraus klar hervor, dass er diese Erscheinung als 'Chlorophyllwirkung ohne Chlorophyll' andeutet.

Im selben Jahre publizierte der heutige Altmeister der Mikrobiologie, Sergei Winogradsky [1887, 1888] seine grundlegenden Studien über den Stoffwechsel der Schwefelbakterien. Winogradsky schloss aus seinen Beobachtungen, dass für bestimmte farblose Organismen wie *Beggiatoa* und *Thiothrix* die Oxydation von Schwefelwasserstoff zu Schwefelsäure die energieliefernde Reaktion, d.h. die Atmungsreaktion, bildet. Die Assimilationsbedürfnisse dieser Organismen sollten jedoch befriedigt werden von der sehr geringen Menge der im Wasser der Schwefelquellen vorkommenden organischen Substanz.

Die Hypothesen von Heraeus und von Hueppe bezüglich der Phy-

siologie der nitrifizierenden Bakterien haben dann aber offenbar einen starken Eindruck auf Winogradsky [1890, 1891] gemacht. Mit grosser Energie hat er sich auf das Studium der nitrifizierenden Bakterien geworfen und im J. 1890 erscheint die entscheidende Publikation, worin das Vermögen zur Kohlensäureassimilation für diese Organismen ausser Zweifel gesetzt wird. Gleichzeitig wird dieses Prinzip dann auch auf die früher studierten Schwefelbakterien und auf die inzwischen ebenfalls aufgestellte physiologische Gruppe der Eisenbakterien übertragen.

Hiermit war also die erste Bresche in die 'Heiligkeit' des Kohlensäure-Moleküls geschlagen. Andererseits hatte die dadurch im primären Bild unerlässlich gewordene Änderung einen keineswegs umstürzenden Charakter. Man hatte die existierenden Ansichten nur in der Weise zu amendieren, dass nicht nur die vom Chlorophyll absorbierte Lichtenergie, sondern auch die chemische Energie hohen Potentials, welche bei der in biochemischer Hinsicht so einzigdastehenden Oxydation von anorganischen Substraten, wie NH_4 , NO_2 , H_2S , Fe'' u. dgl. zustandekommt, imstande war eine Reduktion der Kohlensäure zu bewirken.

Die Annahme scheint berechtigt, dass das auf diese Weise amendierte Bild sich während etwa 30 Jahren praktisch ungeändert behauptet hat. Die Kohlensäure schien eine Verbindung zu sein, welche im Naturgeschehen unangreifbar war, so lange sie nicht in grüne Zellen oder in Zellen mit chemo-autotrophem Stoffwechsel eintrat.

In späteren Jahren hat sich nun diese Lage nicht unerheblich geändert und besonders in der Welt der Mikroorganismen sind mehrere abweichende Fälle von Kohlensäureangriff bekannt geworden.

Die Feststellung der Kohlensäureassimilation durch die Purpurschwefelbakterien muss in diesem Zusammenhang wohl zuerst erwähnt werden. Vermutungen, dass auch diese Organismengruppe hierzu befähigt war, waren schon von Engelmann i. d. J. 1885 und 1888 ausgesprochen worden. Doch waren derartige Ansichten ebenfalls heftig bekämpft worden. Was war doch der Fall?

Für die älteren Physiologen war der Gedanke einer Kohlensäureassimilation unabweisbar verknüpft mit der Ansicht, dass die Kohlensäure einer Umlagerung unterliegen würde, wie diese in den grünen Zellen bei Licht stattfindet, nämlich:



Nun ergab sich jedoch die Schwierigkeit, dass alle Versuche, auch im Stoffwechsel der Purpurschwefelbakterien die Bildung von Sauerstoff nachzuweisen, fehlschlügen. Dazu kam, dass Winogradsky [1888] aus gutem Grunde nicht nur für die farblosen Schwefelbakterien, sondern auch für die genannte Organismengruppe die Oxydation von Schwefelwasserstoff zu Schwefelsäure als Quelle der Lebensenergie angedeutet hatte. Die Schwierigkeit, dass die Purpurbakterien in der Natur vorwiegend an Orten vorkommen, wo der Luftsauerstoff keinen oder nur einen sehr beschränkten Zutritt hat, wurde aufgehoben durch die Annahme einer Symbiose mit gleichzeitig anwesenden grünen Zellen.

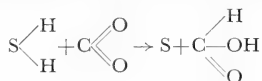
Auch die von Buder [1919, 1920] ausgeführten schönen Untersuchungen über die Stoffwechselphysiologie der Purpurschwefelbakterien konnten den inneren Widerspruch, welcher den bis dahin gegebenen Vorstellungen anhaftete, nicht restlos beseitigen. Wohl ging aus Buder's Beobachtungen deutlich hervor, dass wenigstens unter den gewählten Bedingungen für die Vermehrung der betreffenden Bakterien sowohl Licht als auch Schwefelwasserstoff unentbehrlich waren.

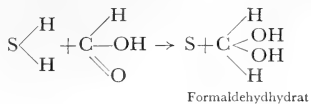
Eine Lösung der diesbezüglichen Fragen wurde dann 1931 von meinem früheren Mitarbeiter Van Niel [1931] erbracht. Auf Grund von in Delft angefangenen, aber erst in Kalifornien systematisch durchgeführten Versuche konnte Van Niel die Lage auf der Basis der Dehydrierungstheorie der Kohlensäureassimilation aufklären.

Als Ausgangspunkt seiner Ansichten diente die Vorstellung, dass die Kohlensäureassimilation in den grünen Zellen als eine gekoppelte Dehydrierung und Hydrierung betrachtet werden muss, gemäss der Gleichung:



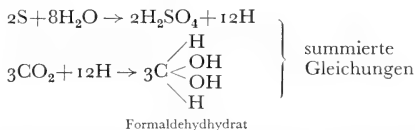
welche Vorstellung u.a. besagt, dass der freiwerdende Sauerstoff dem Wasser und nicht der Kohlensäure entstammt. Van Niel betonte nun die Möglichkeit den Stoffwechsel der grünen und der Purpurschwefelbakterien auf völlig analoge Weise zu betrachten unter Annahme, dass hier primär die eintretende photochemische Reaktion der Gleichung





entsprechen würde.

Bei den Purpurschwefelbakterien würden sich dann Dehydrierungen des Schwefels zu Schwefelsäure mit weiterer gleichzeitiger Kohlensäurehydrierung anschliessen:



Diese Ansichten wurden dann von Van Niel auf überzeugende Weise experimentell bestärkt, indem er zeigte, dass die Menge der von den grünen Bakterien und von Purpurbakterien photochemisch verarbeiteten Kohlensäure tatsächlich von der Menge des vorhandenen Schwefelwasserstoffs bedingt war.

Durch diese Untersuchungen gesellten sich die grünen und roten Schwefelbakterien, angesichts der Tatsache, dass sie ebenfalls zur Kohlensäureassimilation befähigt sind, zu den grünen und chemoautotrophen Zellen.

Ganz revolutionär vom Standpunkte des Kohlenstoffkreislaufes war aber auch dieser Fortschritt nicht: auch hier blieb die Lichtenergie der entscheidende Faktor und ausserdem hat es sich herausgestellt, dass die respektivisch in den grünen und in den roten Bakterien wirkenden Pigmente, das Bakterioviridin und das Bakteriochlorophyll, dem eigentlichen Chlorophyll sehr nahe verwandt sind. Jedenfalls darf konstatiert werden, dass in diesem Falle der Sinn der Kohlensäureassimilation ganz durchsichtig blieb: auch hier steht sie im Dienste des Aufbaus der organischen Substanz.

In dieser Hinsicht traten nun aber merkwürdige Komplikationen auf. Van Niel betonte in seiner Arbeit, dass die bis jetzt bekannten Typen von photochemischer Kohlensäureassimilation nur Spezialfälle eines allgemeinen Vorganges bilden, welcher durch die allgemeine Gleichung:



wiedergegeben wird und worin H_2A sehr verschiedene Wasserstoffdonatoren repräsentiert.

Tatsächlich gelang es Van Niel nachzuweisen, dass bei der photochemischen Kohlensäureassimilation der Purpurschwefelbakterien auch reduzierte Verbindungen wie Leukomethylenblau, aber auch mehr physiologische organische Verbindungen, wie Laktate und Pyruvate, als Wasserstoffdonatoren fungieren können. Von meinem Mitarbeiter Muller [1932] wurden dann diese vorläufigen Beobachtungen vollauf bestätigt und in verschiedener Hinsicht erweitert. Auch Succinate, Malate, Acetate und Butyrate erwiesen sich als durchaus brauchbare Wasserstoffdonatoren für die photochemische Kohlensäureassimilation der Purpurschwefelbakterien.

Man wird sich hierbei fragen, aus welchen Gründen denn eigentlich in diesen Versuchen auf eine Kohlensäureassimilation geschlossen wurde. In den meisten Fällen liess sich experimentell nur feststellen, erstens dass die Entwicklung der Bakterien in einem Nährmedium, welches neben Bicarbonat eines der genannten organischen Substrate enthielt, streng an das Licht gebunden war, und weiter dass diese Entwicklung von einem Verbrauche des Substrats und einer Produktion von Kohlensäure begleitet war. Und zwar wurde um so mehr Kohlensäure erhalten, je nachdem das Substrat im Vergleiche zum Kohlenhydrat stärker oxydiert war. Dass dennoch Kohlensäure tatsächlich in das Reaktionsgeschehen einbezogen wurde, ergab sich aus der Beobachtung, dass in Abwesenheit von Kohlensäure beim Anfang des Versuches die Entwicklung ausblieb.

Wichtiger in dieser Hinsicht war jedoch die Beobachtung, dass bei Verwendung von Substraten, welche im Vergleiche zum Kohlenhydrat reduziert waren, wie z.B. Butyrat, die Entwicklung der Bakterien den theoretischen Erwartungen gemäss, mit einem nicht unerheblichen Kohlensäureschwunde verbunden war.

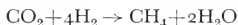
Es ist ohne weiteres klar, dass hier der ursprüngliche Sinn der Kohlensäureassimilation – der Aufbau der organischen Substanz – völlig verloren gegangen ist. Ist doch hier die Synthese mit der Zerstörung organischer Substanz, öfters sogar unter Rückbildung von Kohlensäure als Produkt einer erschöpfenden Dehydrierung, verknüpft.

Wie unerwartet diese Feststellungen auch sein mögen, man hat sich doch immer klar zu machen, dass es sich hierbei stets um Vorgänge handelt, welche gewissermassen als Aberrationen der Lichtwirkung

interpretiert werden können. Es hat den Anschein, als ob das Licht sich bei Abwesenheit der natürlichen Wasserstoffdonatoren, d.h. bei Abwesenheit der anorganischen oxydierbaren Schwefelverbindungen, an andere Wasserstoffdonatoren vergreift und dadurch die beschriebenen mehr oder weniger unphysiologischen Vorgänge hervorruft.

Doch ist die 'Entheiligung' der Kohlensäure seitdem noch viel weiter fortgeschritten. Viel schlimmer von unserem Standpunkte aus sind die in den letzten Jahren bekannt gewordenen Fälle, in welchen die Kohlensäure auch im Dunkeln verschwindet, und dies ohne dass – wie bei den chemo-autotrophen Zellen – die hochwertige Energie anorganischer Oxydationen als 'Entschuldigung' angeführt werden kann.

Vor dreissig Jahren hatte Söhngen [1906] schon gezeigt, dass die Bakterien der Methangärung der fettsauren Salze imstande sind die Reaktion:



zu bewirken. Bei einer Fortsetzung dieser Versuche entdeckte sein Mitarbeiter Wieringa [1936], dass auch andere obligat anaerobe sporenbildende Bakterien Kohlensäure und Wasserstoff verarbeiten und zwar in Essigsäure umsetzen können.

Etwa zur gleichen Zeit machte Woods [1936] die sehr bemerkenswerte Beobachtung, dass *Bacterium coli* nicht nur imstande ist die Ameisensäure in Kohlensäure und Wasserstoff zu spalten, sondern ebenfalls die umgekehrte Reaktion herbeiführen kann, sodass von beiden Seiten ausgehend ein Gleichgewichtszustand erreicht werden kann.

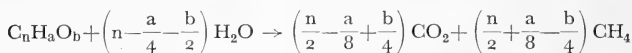
In allen zuletzt genannten Versuchen gilt nun der mildernde Umstand, dass gasförmiger Wasserstoff wohl eine sehr besondere Nahrungskomponente ist und man könnte sich denken, dass wenn auch dieser Faktor ausgeschaltet sein würde, die Kohlensäure in den Zellen mit heterotrophem Stoffwechsel ihre Existenz doch unbedroht fortsetzen könnte.

Doch hat es sich unmöglich gezeigt, auch diese letzte Aussage aufrechtzuerhalten. In dieser Hinsicht ist ein Umsturz hervorgegangen aus Untersuchungen, welche der amerikanische Bakteriologe Barker [1936, 1937] in meinem Laboratorium über den internen Chemismus der sogenannten Methangärung angestellt hat.

Wir verdanken Hoppe-Seyler, Omelianski und vor allem Söhngen

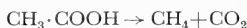
den Nachweis, dass es möglich ist, sehr verschiedene organische Substanzen einer Umlagerung zu unterwerfen, welche dadurch gekennzeichnet ist, dass der Kohlenstoff praktisch quantitativ in Kohlensäure und in Methan zurückgefunden wird.

Von Buswell und seinen Mitarbeitern [Boruff and Buswell, 1929; Neave and Buswell, 1930; Symons and Buswell, 1933] wurden diese Ergebnisse in den letzten Jahren noch sehr wesentlich erweitert und ihre Gültigkeit auf sehr verschiedene Substrate erstreckt. Dies führte Buswell zu der Aufstellung einer ganz allgemeinen Formulierung dieser Vorgänge:

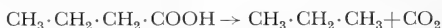


Diese Formulierung wurde auf Grund sehr zahlreicher Beispiele experimentell ausnahmslos bestätigt.

Bis vor kurzem war jedoch das Wesen dieser sogenannten Methangärung völlig rätselhaft geblieben. In Spezialfällen konnte man sich noch einigermaßen eine Vorstellung über diesen Vorgang machen. So war es verführerisch die Methangärung der Essigsäure gemäss der Gleichung



als einen einfachen Dekarboxylationsvorgang aufzufassen. Doch diese Vorstellung versagte in anderen Fällen völlig, wurde doch bei der Vergärung von Buttersäure in Analogie hiermit keineswegs Propan gebildet, wie aus der Gleichung



hervorgehen würde. Im Gegenteil auch bei der Vergärung der Buttersäure entsteht quantitativ Methan neben der Kohlensäure, gemäss der Gleichung:



Eine weitere sehr bemerkenswerte Eigentümlichkeit sämtlicher Methangärungen ist nun, dass dabei niemals neben dem Methan auch nur die geringsten Spuren irgendwelcher sonstigen Reduktionsprodukte der Substrate angetroffen worden sind.

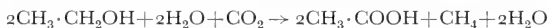
Diese Umstände, zusammen mit der schon erwähnten alten Feststellung Söhngens bezüglich der Fähigkeit der Methanbakterien Kohlensäure mit gasförmigem Wasserstoff zu reduzieren, hat nun

Van Niel veranlasst die kühne Hypothese aufzustellen, dass auch bei der Methangärung der organischen Substrate diese nur als Wasserstoffdonatoren bei der Hydrierung gleichzeitig anwesender Kohlensäure fungieren würden.

Barker hat sich nun zum Ziele gesetzt die Richtigkeit dieses Gedankens experimentell zu prüfen. Von vorneheran war es deutlich, dass dies nur möglich sein würde, falls es gelingen würde die mutmassliche Dehydrierung der Substrate derartig zu gestalten, dass sie unvollständig, d.h. mit Erhaltung des Kohlenstoffskelettes vor sich gehen würde. Einige Aussicht auf diese Möglichkeit war vorhanden auf Grund des einige Jahre früher von meinem Mitarbeiter Baars [1930] erbrachten Nachweises, dass bei der ebenfalls anaerob vor sich gehenden Sulfatreduktion unter bestimmten Bedingungen Alkohole nur unvollständig, d.h. zu den entsprechenden Fettsäuren dehydriert werden.

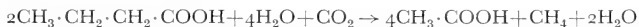
Tatsächlich gelang es nun Barker den experimentellen Nachweis zu bringen, dass analoge Verhältnisse sich auch bei der Methangärung realisieren lassen. Bei der Vergärung von Aethylalkohol in Gegenwart von Kreide, wurde eine kräftige Methangärung erhalten, welche vor allem dadurch gekennzeichnet war, dass das entweichende Gasgemisch fortwährend ärmer an Kohlensäure und reicher an Methan wurde, bis schliesslich fast reines Methan aufgefangen wurde. Die Analyse des Gärgemisches lieferte nun das sehr beachtenswerte Resultat, dass einerseits die Menge der verbrauchten Kohlensäure fast genau derjenigen des gebildeten Methans entsprach und dass andererseits der verschwundene Alkohol quantitativ als sein Dehydrierungsprodukt Essigsäure zurückgefunden wurde.

Hiermit war der merkwürdige Vorgang:

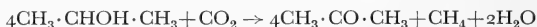


experimentell bestätigt worden.

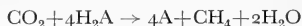
Auch für die Vergärung des n-Butylalkohols liess sich feststellen, dass primär ein völlig analoger Vorgang eintritt, wobei also Buttersäure entsteht. Hierauf schliesst sich dann aber eine weitere Dehydrierung an, wobei das letztgenannte Produkt in Essigsäure übergeführt wird, gemäss der Gleichung



Hierzu kommt dann noch, dass mein Mitarbeiter Schnellen neulich ähnliches für den n-Propylalkohol nachgewiesen hat und ebenfalls die sehr bemerkenswerte Dehydrierung von Isopropylalkohol zu Aceton unter gleichzeitiger Methanbildung feststellen konnte, gemäss der Gleichung:

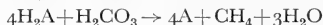
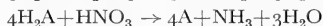
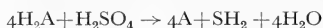


Mit diesen Tatsachen vor Augen scheint es kaum gewagt für die Methangärung die allgemeine Formel:



aufzustellen.

Dieses Ergebnis ist deshalb so erfreulich, weil dadurch die Methangärung als Carbonatreduktion mit den von altersher wohl bekannten Prozessen der Sulfat- und der Nitratreduktion ganz auf eine Linie kommt, wie von den folgenden Gleichungen erläutert wird:



Hierbei ist zu beachten, dass SH_2 , NH_3 und CH_4 die Produkte der erschöpfenden Reduktion der Elemente Schwefel, Stickstoff und Kohlenstoff sind.

Immerhin darf nicht unerwähnt bleiben, dass mit dieser kaum abweisbaren Vorstellung noch Konsequenzen verknüpft sind, welche dem Verständnis grosse Schwierigkeiten machen.

Es versteht sich, dass die gemachte Verallgemeinerung der experimentell festgestellten Ergebnisse u.a. mit sich bringt, dass sie auch für diejenigen Fälle gilt, in welchen die Dehydrierung der organischen Substanz zu Kohlensäurebildung führt.

So sollte z.B. Essigsäure vergären nach der Gleichung:



d.h. auch hier begegnen wir wieder einem Fall, wo die Kohlensäure-reduktion mit einer überwiegenden Kohlensäureproduktion verknüpft ist.

Besonders schwierig wird nun noch die Lage, wenn wir uns klar machen, dass die gegebene Vorstellung fast impliziert, dass die Hydrierung der Kohlensäure zu Methan über verschiedene Reduktions-

stufen führt. Als solche Stufen können wohl kaum andere Körper als HCOOH , CH_2O und CH_3OH in Betracht kommen. Wenn man nun weiss, dass sowohl die Ameisensäure als der Methylalkohol ebenfalls einer Methangärung unterliegen, dann muss man schliessen, dass diese Moleküle in den meisten Methangärungen nur hydriert, aber bei Anwendung als Substrat auch dehydriert werden!

Wie dem auch sei, es ist ohne weiteres klar, dass Barker's Untersuchungen dem Dogma der Unangreifbarkeit der Kohlensäure in heterotrophen Zellen im Dunkeln seine feste Stütze genommen haben. Und für den Kohlenstoffkreislauf ergibt sich, dass wir damit rechnen müssen, dass Kohlensäure verschwinden kann an Stellen, wo man dies auf Grund der klassischen Ansichten für ausgeschlossen halten würde.

Man könnte immerhin noch meinen, dass der Fall der Methanbakterien mehr oder weniger vereinzelt dastehen würde. Tatsächlich lässt sich nicht verneinen, dass es hier eine ganz besondere Bakteriengruppe betrifft; sind doch die Vertreter dieser Gruppe so extrem anaerob, dass eine Reinkultur dieser Organismen, welchen das Vermögen zur Bildung sauerstoffresistenter Sporen abgeht, noch nicht mit Sicherheit durchgeführt werden konnte.

Dass aber auch dieser Vorbehalt nicht stichhaltig ist, geht aus den Versuchen von Wood und Werkman [1936, 1938] hervor, die neulich wohl ausser Zweifel gesetzt haben, dass Propionsäurebakterien bei der Vergärung von Glycerin ebenfalls Kohlensäure in ihren Stoffwechsel beziehen. Und zwar sollen diese Bakterien hierbei eine Synthese eines aus 4 C-Atomen aufgebauten Körpers, nämlich der Bernsteinsäure, bewirken.

Hier begegnen wir also einem weiteren Beispiel, wo die Kohlensäure auch in den heterotrophen Zellen nicht länger unangreifbar erscheint.

Unter diesen Bedingungen fragt es sich, wo dann hier die Grenze gelegen ist und ob wir uns schliesslich nicht damit vertraut machen müssen, dass auch die Zellen bestimmter tierischer Organe Kohlensäure assimilieren können.

Vorläufig kann man noch energische Proteste erwarten, wenn ein Redner ausspricht, dass das Vieh in der Wiese, oder auch sein ganzes Auditorium Kohlensäure assimiliert. Und doch ist die Versuchung hierzu schon heute vorhanden!

Denn es geziemt sich hier einer Umwandlung zu gedenken, die schon seit längerer Zeit zu derartigen Vermutungen Veranlassung gab und bezüglich welcher wir heute mit grosser Sicherheit schliessen dürfen, dass sie tatsächlich mit einer Überführung von Kohlensäure in organische Substanz verknüpft ist. Ich beziehe mich hier auf die Bildung von Harnstoff im tierischen Stoffwechsel. Wir verdanken Krebs und Henseleit [Krebs, 1934] eine genaue Einsicht in die Weise durch welche die Leberzellen imstande sind den Ammoniumstickstoff in Harnstoff überzuführen. Auf Grund der Untersuchungen dieser Forscher dürfen wir jetzt schliessen, dass die Primärreaktion eine Überführung von Ornithin unter Aufnahme von Ammoniak und Kohlensäure in Citrullin ist. Durch weitere Aufnahme von Ammoniak geht letztere Verbindung in Arginin über, welche Substanz dann von der Arginase unter Abspaltung von Harnstoff wieder in Ornithin zurückgeführt wird. Wenn auch nicht vergessen werden darf, dass weder im Citrullin-Molekül noch im Harnstoff-Molekül der Kohlenstoff der aufgenommenen Kohlensäure Veranlassung gegeben hat zu der Bildung von neuen Kohlenstoff-Bindungen, wird man nicht umhin können den genannten Vorgang als eine Neubildung organischer Substanz zu deuten.

Jedenfalls warnt auch das Beispiel der Harnstoffsynthese davor, die Kohlensäure im heterotrophen Stoffwechsel nicht länger als unangreifbar zu betrachten.

Doch ist hiermit das letzte Wort über die Beziehungen zwischen der Kohlensäure und den Zellen mit heterotrophem Stoffwechsel noch keineswegs gesagt. Obgleich die ersten diesbezüglichen Beobachtungen schon vor mehreren Jahren gemacht worden sind, wird merkwürdigerweise in dem besprochenen Zusammenhange fast niemals berücksichtigt, dass aus den neueren Untersuchungen mit grosser Wahrscheinlichkeit hervorgeht, dass die Kohlensäure sogar eine völlig unentbehrliche Nahrungskomponente aller heterotrophen Zellen ist.

Nachdem Nowak [1908] gefunden hatte, dass die Kultur des *Bacterium abortus* wesentlich erleichtert wurde bei gleichzeitiger Kultivierung von *Bacillus subtilis* im selben Raume, und diese Tatsache einer Verminderung der Sauerstoffspannung zugeschrieben worden war, wurde bald versucht diesen Effekt auch auf andere Weise zu erhalten. Dies führte unter anderem dazu den Bang-Bazillus in einer Atmosphäre von Luft, der 10% Kohlensäure zugesetzt worden war, zu kul-

tivieren. Diese Methode erwies sich als besonders erfolgreich und gab Veranlassung zu der Ansicht, dass Kohlensäure eine spezifisch fördernde Wirkung auf das Wachstum der betreffenden Bakterien ausübe. Allmählich wurde diese Ansicht auf sehr verschiedene heterotrophe Bakterien erstreckt. Rockwell und Highberger [1927] gebührt der Verdienst als erste experimentell nachgewiesen zu haben, dass in zahlreichen Fällen das Wachstum sehr verschiedener Mikroorganismen – Bakterien, Hefen und Schimmelpilze – völlig ausbleibt, wenn man die letzten Spuren Kohlensäure aus dem Nährmedium entfernt. Ähnliche Ergebnisse wurden bald auch von andern Forschern berichtet.

So weit bei diesen Versuchen auf festen Nährmedien kultiviert wurde, wurde anfangs versucht bei Anwesenheit kaustischer Soda zur Entfernung der Kohlensäure das Ausbleiben des Wachstums auf ein Austrocknen der oberflächlichen Schichten der genannten Medien zurückzuführen. Diese Möglichkeit wurde jedoch entscheidend widerlegt durch die Feststellung Rockwells und Highbergers, dass das Wachstum in Gegenwart von Chlorcalcium oder von konzentrierter Schwefelsäure nicht oder nur wenig gehemmt vor sich ging.

Die ausführlichen Untersuchungen von Valley und Rettger [1926, 1927], welche sich auf mehr als hundert verschiedene Bakterienarten bezogen, zeigten dann überzeugend, dass es unter gewissen Bedingungen gelingt die Unentbehrlichkeit der Kohlensäure für das Wachstum aller dieser Arten zu beweisen. Doch zeigte es sich, dass zur Erzielung dieses Effektes je nach der zu prüfenden Art verschiedene Massnahmen nötig sind. Dies ist leicht zu verstehen, wenn man in Betracht zieht, dass die Zellen des Impfmateri als im allgemeinen zur Kohlensäurebildung befähigt sein werden und deshalb die Intensität, womit die Kohlensäure absorbiert wird, entscheidet, ob in den Zellen tatsächlich Kohlensäuremangel entsteht. Dass die mehr oder weniger grosse Reichhaltigkeit des Nährmediums und auch das pH dieses Mediums in dieser Hinsicht von Bedeutung sein werden, lässt sich leicht einsehen.

Winslow, Walker und Mitarbeiter [1932], Walker [1932], und später auch Gladstone, Fildes und Richardson [1935], haben weiter gezeigt, dass die Unentbehrlichkeit der Kohlensäure sich unter gewissen Umständen ebenfalls bei Kultur in flüssigen Medien demonstrieren lässt. Hierzu vergleicht man Kulturen, welche mit gewöhn-

licher Luft aeriert werden, mit solchen wobei die verwendete Luft mittels Kalilauge vorher von den letzten Spuren Kohlensäure befreit worden ist. Bei schwacher Impfung und bei Anwendung von Nährmedien, welche ein nur schwaches kohlenensäurebindendes Vermögen haben, bleibt im letzten Falle jegliches Wachstum aus, während in den normal aerierten Kulturen die in geringer Konzentration vorhandene Kohlensäure ihre wachstumsauslösende Wirkung deutlich manifestiert.

Auch mein Mitarbeiter Hes [1938] konnte diese Ergebnisse vollauf bestätigen. Der von befreundeter Seite erhobene Einwand, dass bei einer derartigen Versuchsanstellung das Ausbleiben des Wachstums möglicherweise nicht dem Kohlensäuremangel, sondern der wachstumhemmenden Wirkung flüchtiger, der Lauge entstammenden Substanzen zugeschrieben werden müsste, wurde neulich in meinem Laboratorium von Tak beseitigt. Dieser stellte nämlich fest, dass die mittels Lauge kohlenensäurefrei gemachte Luft sofort wieder ihre befruchtende Wirkung erhielt, wenn sie nachher noch eine Waschflasche mit verdünnter Bicarbonatlösung passierte.

Hes benutzte bei seinen Versuchen teilweise eine etwas abweichende Methodik. Er konnte nämlich das Ausbleiben des Wachstums infolge des Kohlensäuremangels ebenfalls nachweisen in Kulturen in flüssigen Medien ohne Luftpassage, falls er die Kultur in geschlossenem Raume vornahm, die Gasatmosphäre in Kontakt mit kaustischer Soda brachte und die Kulturflaschen während des ganzen Versuchs schüttelte. Unter diesen Bedingungen blieb auch nach neun Tagen jegliches Wachstum aus, wobei in Parallelversuchen das bemerkenswerte Ergebnis erhalten wurde, dass nach einer erst nach 6 Tagen vorgenommenen Entfernung der Lauge sofort wieder Wachstum eintrat.

Es fragt sich nun, welche Ursachen dem hier beschriebenen, so merkwürdigen Phänomen zugrunde liegen. Man könnte hierbei an die folgenden Möglichkeiten denken.

An erster Stelle ist die Vermutung geäußert worden, dass die Kohlensäure lediglich durch ihren pH-erniedernden Einfluss auf das Medium wirksam sein könnte. Die Ansicht darf heute wohl als endgültig widerlegt betrachtet werden. Erstens kann der Einfluss der Spuren Kohlensäure auf die Wasserstoffionenkonzentration der fast immer sehr merkbar gepufferten Medien nur sehr gering sein. Weiter ist in vielen Fällen festgestellt worden, dass sich der Kohlen-

säureeffekt auf das Wachstum bestimmter Bakterienarten innerhalb weiter Grenzen des pH-Gebietes manifestiert und zwar ist die unterste Grenze so niedrig, dass es völlig ausgeschlossen ist, dass die Kohlensäure in dem mehr nach der alkalischen Seite verschobenen Medium das pH unter diesen Minimalwert bringt.

Eine zweite Erklärungsmöglichkeit wäre, dass die Kohlensäure ein unentbehrlicher Baustein für den Aufbau bestimmter Komponente des neuen Zellmaterials ist. Dies würde heissen, dass die Kohlensäure wie bei den chemo-autotrophen Organismen auch in den heterotrophen Zellen in eine Synthese einbezogen würde. Wenn man sich überlegt, welche primären Reaktionsprodukte in diesem Falle aus der Kohlensäure entstehen könnten, so kann man kaum umhin zu schliessen, dass entweder als erstes Reduktionsprodukt Ameisensäure entstehen muss, oder dass die Kohlensäure mit Ammoniak unter Bildung von Harnstoff reagiert. Die von Hes vorgenommenen Versuche, um die Richtigkeit dieses Gedankenganges experimentell zu prüfen, lieferten aber ein völlig negatives Resultat. Der typische Kohlensäureeffekt liess sich weder durch Zugabe von Ameisensäure, noch durch Zugabe von Harnstoff an das Medium unterdrücken, wie man doch auf Grund der aufgestellten Hypothese erwarten sollte.

Unter diesen Umständen ist man geneigt die schwerwiegenden Folgen der Kohlensäureentziehung auf die Wachstumsfähigkeit der Zelle auf eine Änderung des Quellungsgrades bestimmter biologisch wichtiger Eiweissysteme zurückzuführen. Man findet diesen Gedanken ausgesprochen in einer Arbeit von Rippel und Heilmann [1930], welche Forscher die grosse Bedeutung geringer Kohlensäuremengen für die Keimung der Sporen von *Aspergillus niger* feststellten.

Falls eine derartige Erklärung zutreffen würde, lässt sich nun aber erwarten, dass der 'Kohlensäureeffekt' sich nicht nur auf das Wachstum der Zellen geltend machen würde, sondern auch auf die biochemischen Eigenschaften einer präformierten Bakterienmasse. Hes hat sich nun die Aufgabe gestellt diese Möglichkeit einer experimentellen Prüfung zu unterziehen. Auf Grund einer im Institut Pasteur zu Paris unternommenen Versuchsreihe hat er in einer vorläufigen Mitteilung berichtet in dieser Richtung positive Erfolge erzielt zu haben. Hes meint festgestellt zu haben, dass auch die Methylenblau-reduktion durch sogenannte 'resting bacteria', welchen Vorgang er nach der üblichen Thunbergschen Methodik untersuchte, bei sorgfältiger

Entziehung der letzten Spuren Kohlensäure völlig gehemmt, oder doch jedenfalls erheblich verzögert wird.

Es wäre verfrüht auf Grund dieser vorläufigen Angaben weitgehende Schlussfolgerungen zu ziehen. Wenn die Hesschen Beobachtungen sich bestätigen lassen, würde man immerhin noch mit verschiedenen Möglichkeiten zu rechnen haben. Es wäre tatsächlich möglich, dass die durch Kohlensäureentziehung bewirkte Hemmung auf eine Entquellung der Eiweisskomponente bestimmter Fermente zurückzuführen sei. Eine andere Möglichkeit wäre jedoch, dass sowohl in den Methylenblauversuchen, wie auch bei dem für das Wachstum unentbehrlichen Atmungsvorgange, das Oxydoreduktionssystem: Kohlensäure-Ameisensäure ein Glied der betreffenden Reaktionsketten bildet. Eine derartige Möglichkeit ist von Krebs [1937] und von Elsdon [1938] aufgeworfen worden auf Grund von Beobachtungen bezüglich des Einflusses der Kohlensäure auf bestimmte von *Bacterium coli* bewirkte Stoffwechselprozesse. Diese Ansicht scheint mir jedoch nicht sehr wahrscheinlich, weil der 'Kohlensäureeffekt' auf das Wachstum sich bei den verschiedensten heterotrophen Zellen geltend macht, während es für viele dieser kaum annehmlich ist, dass sie zur Einstellung eines Kohlensäure-Ameisensäure-Gleichgewichtes befähigt sein werden.

Wenn auch wir gestehen müssen, dass die tieferen Ursachen der Kohlensäure-Unentbehrlichkeit für den Stoffwechsel der heterotrophen Zellen noch völlig ins Dunkle gehüllt sind und eine Lösung dieser Frage der künftigen Forschung vorbehalten bleiben muss, wird die Realität des Phenomens hierdurch nicht angegriffen.

Klar tritt auch hierdurch die Unzulässigkeit einer Teilung des Kohlenstoffkreislaufes in eine nur Kohlensäureproduzierende und eine nur Kohlensäurekonsumierende Hälfte hervor.

Wenn wir am Ende unserer Ausführungen das gesamte Gebiet noch einmal überblicken, müssen wir unumgänglich schliessen, dass vom anfänglich skizzierten biologischen Weltbilde nicht viel übriggeblieben ist. Angesichts der ursprünglich verteidigten 'Heiligkeit' des Kohlensäuremoleküls fühlt man sich wie ein Bilderstürmer.

Demgegenüber steht dann der Gewinn an Erkenntnis und das daraus hervorgehende Bedürfnis an tiefergehende wissenschaftliche Forschung. Wenn die hier gegebenen Betrachtungen dazu führen würden, dass auch die noch junge, aber schon rühmlichst bekannte

finnische biochemische Schule sich entschliessen würde, sich an diesen Forschungen zu beteiligen, so wäre dies für mich ein Grund grösster Genugtuung.

Und was schliesslich die Kohlensäure selbst anbelangt, wenn auch ihre ursprüngliche Stellung im biochemischen Geschehen sich nicht hat behaupten können, bedeuten die neueren Forschungen auch für sie gewissermassen eine Rehabilitation. Während Plinius sie bald nach dem Anfange unserer Zeitrechnung als '*spiritus letalis*' deutete, liegt, angesichts der ans Licht getretenen Unentbehrlichkeit dieses Gases für alles was lebt, vielmehr Grund vor, ihr den Namen zu schenken von:

Spiritus vitalis.

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THREE DECADES PROGRESS IN MICROBIOLOGY

It is only appropriate that our first thoughts this afternoon go out to that pioneer in the field of microbiology whose birthday, now 104 years ago, we commemorate today. In these surroundings it may be deemed superfluous to pause upon the many important contributions Emil Chr. Hansen has made to pure and applied microbiology during his well-used life-time. There is, however, every reason for the lecturer to acknowledge his great debt of gratitude towards the man who in his last will took measures to promote even after his death the development of his beloved science for long times to come. In saying this I do not wish to suggest that the superficial considerations which I have the honour to present before you this afternoon will influence in the least the growth of microbiology. But I want to give expression to my conviction that owing to the contact with so many distinguished representatives of Danish science I shall be on my returning home, if not a better man, at least a better scientist. And it is also at this international diffusion of knowledge that Hansen must have aimed, when he decided to call into existence the 'Fond' which bears his name.

Having gone back in our minds to the year 1909, incidentally the year in which my own initiation in the world of microbes took place, it is tempting to take a retrospective view of the development of microbiology as a science, and to compare the present situation with the stage reached about thirty years ago. Such a survey can, of course, not aim at completeness, and I shall have to confine myself to a passing into review some of the major trends of investigation which on looking backward come to the fore. In doing so I hope to bring into relief the considerable progress made in three decades of almost restless research, a progress which is characterized by an ever-increasing recognition of the unity that is at the basis of the at first sight so bewildering diversity in the various manifestations of microbe life.

Let us now first dwell for a moment on the stage of development

microbiology had reached in the period immediately after Hansen's decease.

Seen from the distance of to-day it is clear that microbiological science passed through a phase of transition then. The glorious period of exploration of the microbe world had practically come to an end. The preceding quarter of a century had witnessed an almost endless series of triumphs in the discovery and isolation of numerous microbial species. In the hands of masters like Winogradsky and Beijerinck the principle of the elective culture had opened the world of invisible life. Owing to the so pronounced diversity in nutritional requirements of the various types of micro-organisms it proved possible to bring about accumulations of many diverse microbes, thus facilitating their isolation and a first investigation of their properties. By these studies a clear light was thrown on the almost ubiquitous occurrence of numerous microbes on earth. But still more impressive was the manifestation of the extreme diversity of chemical systems which proved to be able to support microbial life. I have only to remind you of Winogradsky's great discovery of the so-called autotrophic bacteria characterized by their ability to proliferate in fully inorganic media in the absence of radiant energy. Other forms of life again were found which satisfied their nutritional demands with single organic compounds either considered to be almost chemically inert like the paraffin hydrocarbons, or even known to be harmful for the greater part of microbes like phenols. In short it can be said that one specialist amongst the microbes was hardly discovered, or already a second specialist with still greater achievements was being announced. The microbiological scenery seemed to be one grand scientific 'Tivoli'.

However, around 1910 the activity in this direction had obviously slackened: the great explorers of the microbe world had done their job efficiently. One would go too far by stating that since then no new species have been discovered. But how poor has the harvest been in later years when compared with that in the earlier period. And this notwithstanding the enormous increase in the number of 'microbe-hunters' during the last three decades. Is it not characteristic that the almost incredible extension of the study of bacteriology in the 'New World' since the first world war has not at all led to the discovery of a 'new microbe world'? It is true that the number of micro-organisms annually described as new species is still alarmingly large. But how

many of these species can rightly be claimed as such, and if so cannot they nearly always be connected with other well-known forms, from which they only differ in secondary characters?

It will be clear that the conclusion of the exploration period in microbiology did not put an end to the growth of this science. It only meant that new trends of investigation manifested themselves, all tending to consolidate the occupied territory by gaining a deeper insight into the properties of the newly discovered world. Evidently a first necessity hereto was to replace the chaotical conglomerate of known living forms by a more or less systematic arrangement. In first instance this asked for a recognition and evaluation of natural relationships between the various forms, and the demarcation of natural groups as building stones for a satisfactory classification.

If for a moment we restrict ourselves to the bacteria, we have to acknowledge that the older workers too had already made several efforts to arrive at some system of classification. But these systems all had the limitation that they were based solely on the scanty morphological data derived from the microscopical examination of the organisms in question.

It is now the great merit of the nestor of Danish bacteriologists, Orla-Jensen, to have introduced a new era in the study of bacteriological classification. In several papers Jensen has made an eloquent and broadly documented plea that physiological characters should determine the main lines of a bacterial system. In their entirety Jensen's views have not been accepted, and yet they mark a milestone in the development of bacterial taxonomy. For they have opened the eyes of generations of bacteriologists to the truth that, given the enormous diversity of metabolic properties amongst bacteria which contrasts so sharply with the relatively great uniformity characteristic of the metabolism of higher plants and animals, it is not only permissible, but even imperative also to take the physiological properties into account in the ordering of the bacterial kingdom. For those bacteriologists who have not yet been reconciled with this idea it may be remarked that nowadays there is increasing evidence that differences in catabolic properties of bacterial cells can be considered as expressions of variations in submicroscopical morphology. Anyhow, there is no doubt that Orla-Jensen's views have greatly influenced the numerous

proposals for bacterial classification put forward after 1910. It is true that we still are far from an in all respects satisfactory system, nor can we hope that such an aim will ever be attained. But nevertheless, it can be stated that the phylogenetic approach to the solution of this problem which Stanier and Van Niel have brought in their recent study, has thrown a clear light on the interrelationships of the main bacterial groups, and their place in the natural kingdom. And it seems likely that a future more detailed system will be based on the same judicious balance in the evaluation of both morphological and physiological properties which is characteristic of the study of the American authors.

A second feature of the tendency to bring order into the world of microbes has been the foundation of several institutions where collections of living micro-organisms have been brought together and are maintained. As such mention may be made of the 'Centraalbureau' for cultures of fungi, including yeasts, founded already in 1906 in my own country, the National Collection of Type Cultures established at the Lister Institute in London, the American Type Culture Collection formerly in Chicago, now in Washington, and the new, economically very important collections of moulds and yeasts established in the Northern Regional Research Laboratory at Peoria (Ill.). Moreover, several scientists who have been specializing on certain groups have maintained private collections, and it should be mentioned here that investigators in demand of cultures of lactic acid bacteria will seldom have appealed in vain to Orla-Jensen's generous cooperation.

It seems certain that collections like those mentioned serve several useful purposes. In the first place they promote the dissemination of knowledge by providing scientists all over the world with authentic cultures of organisms discovered or described by their colleagues elsewhere. Secondly these institutions often develop into centres where special groups of organisms are more or less exhaustively studied from both a systematic and a physiological standpoint. This not rarely leads to the publication of monographs dealing with such groups, of which I need only mention Orla-Jensen's two volumes dealing with 'The Lactic Acid Bacteria' in order to impress the importance of such studies upon you.

Looking back once more into the post-explorative era of microbiology

other trends of investigation besides that of ordering and classification are easily discerned.

In the foregoing I have already emphasized the diversity in metabolism characteristic for the various types of micro-organisms, and it is only self-evident that the often quite amazing chemical activities of many microbes has led to attempts to penetrate deeper into the secrets of their metabolism. In order to give you some idea of the achievements attained along this line during the last three decades I should remind you that in metabolism two counterparts can and should be distinguished, *viz.*, anabolism and catabolism. The term anabolism comprises the processes leading to the building up of cell constituents, while we designate with catabolism those processes which are only of significance to the cell from the standpoint of energy supply.

As for the catabolic processes in higher organisms, plants as well as animals, it has been known since Lavoisier's fundamental observations that they have to be considered as a slow combustion process of the carbohydrate constituents of the food, and that, therefore, they are characterized by a consumption of oxygen and a simultaneous production of an equivalent amount of carbon dioxide. This so-called respiration process has consequently long been considered to be an essential attribute of life.

Already a first survey of microbial life did bring to light that herein this uniformity in catabolism so characteristic of the higher forms of life is absent. In the first place it soon became evident that, although the proliferation of many microbes is indeed dependent on the availability of free oxygen, the respiration processes differed widely in the nature of the compounds acceptable as substrates for the oxidation process. Moreover, with many types of microbes this process did not yield the final oxidation products carbon dioxide and water, but led to the excretion of incomplete oxidation products in the medium.

Even more startling was the observation made by Pasteur already in 1860 that there were several microbes which not only could thrive in the absence of oxygen, but for which this gas even was decisively harmful. It did, however, not escape Pasteur's attention that this unexpected observation correlated with the occurrence of other chemical conversions known from time immemorial as fermentation (or putrefaction), and his genius made him at once conclude to the physiological equivalence of respiration and fermentation.

Now it gradually became evident that the fermentation processes brought about by various types of micro-organisms mutually show great differences. Although they may attack the same substrate, the products of the conversion often differ considerably. While in the best-known fermentation process, the conversion of sugar into carbon dioxide and ethyl alcohol, the products are stoichiometrically related both mutually and to the sugar fermented, in many other cases in which numerous fermentation products are formed such relations do not exist. Moreover, the quantities in which these products are formed may vary largely owing to differences in the external conditions to which the fermenting organism is exposed.

Altogether, a quarter of a century ago the biochemistry of catabolic processes in the microbe world showed such an extreme diversity that at that time one might well despair of any attempt to elucidate this superchemistry.

It is undoubtedly one of the greatest results of three decades of microbiological investigation that nowadays this aspect has so fundamentally changed. If we try to survey the causes which have led to this revolution it is at once clear that we owe this progress mainly to the splendid investigations of both Meyerhof and Warburg and their co-workers. Concentrating their efforts on an analysis of the processes of alcoholic fermentation and glycolysis, and building on the work of pioneers like Harden and Neuberg, they have been able to give us a clear insight into these processes.

Stimulated by these so successful investigations other types of fermentations have likewise been more or less intensively studied in several other laboratories. The chief result of all this has been that the so amazing diversity of the various fermentation processes has given way to a quite unexpected unity in principle.

The main points of the insight gained can be summarized as follows.

All these fermentation processes consist of a series of individual step-reactions, each of which constitutes a chemically intelligible, and extremely simple type of reaction, the greater part of these step-reactions being typical equilibrium reactions. Moreover they show great uniformity in so far that many of them can be characterized as reactions in which hydrogen atoms (or electrons) are transferred from one constituent to another.

Another feature of our present insight into the fermentation proc-

esses is, however, the important rôle which phosphoric esters play therein. Not only must we accept that the first conversion of the carbohydrate to be fermented is the formation of a hexosephosphoric acid, but it has also become increasingly clear that a transference of the phosphate group from one intermediate product to a second one, ultimately leading to a closed phosphate cycle, is an essential counterpart of the hydrogen transference just mentioned. The more so, because at least in several cases a direct linkage between the two types of processes has been proved to exist.

Especially gratifying is the result that in all these fermentation processes the first steps of the breakdown of the carbohydrate have been proved to be the same, and that only in the final stages of this breakdown process specific step-reactions take place which are responsible for the differences in the final fermentation products.

Still more important is that these attempts to arrive at a unification of microbial catabolism have not remained restricted to the typical cases of sugar fermentation. In this connexion I mention only that also for the long known bacterial processes of denitrification, sulphate reduction and methane fermentation it has been shown that they represent typical cases of catalytic hydrogen transference in which respectively nitrates, sulphates and carbonates act as hydrogen acceptors.

The greatest triumph of this analysis of microbial catabolism is, however, that it has proved possible to extend these unifying efforts also to the various types of respiration processes encountered in the microbe world. Numerous investigations initiated by Wieland's classical observations leave no doubt that these processes too consist of a series of step-reactions of the hydrogen or electron transference type, the free oxygen acting as the final hydrogen acceptor. This, of course, is a result with wide implications. For it means that at the bottom of the physiological equivalence of respiration and fermentation as advocated by Pasteur there is a conformity in essence which is highly edifying to the scientific mind. It is clear that hereby new perspectives have been opened for a satisfactory understanding of the interrelation between fermentation and respiration in one and the same type of cell as manifested in the so-called reaction of Pasteur. But the unification in question also opens the way for a better appreciation of evolutionary developments which have taken place in the microbe world, since the

antithesis between the aerobic and anaerobic mode of life has been largely removed.

The question now arises in how far three decades of biochemical investigations which have added so much to our insight into microbial catabolism have also succeeded in elucidating the anabolic counterpart of metabolism. Even a superficial survey of anabolism suffices to recognize that the task of the biochemist in this respect is tremendous. The building up of the thousands of chemically widely differing cell constituents starting from the compounds of the food is especially in the microbe world an extremely impressive problem, for numerous are the instances in which microbes are performing this miracle in media in which the number of the organic constituents is restricted to one or two. This obviously means that proliferation of a micro-organism in such a medium involves a super-chemistry far beyond the imagination of the boldest and cleverest of our organic chemists.

Nevertheless we may state that in this domain too real progress has been made in the period under consideration. Much of this progress is closely linked up with the advances made regarding our knowledge of catabolic processes. This becomes at once clear when we first consider what may be called the main aspect of anabolism.

Thirty years ago the view was generally held that in anabolism we are dealing with a very special type of chemistry. For it is of common experience that in these anabolic processes often compounds are formed with an energy content which greatly surpasses that of the substrate of the synthesis. I need only remind you of the formation of fat by cells which develop in a medium with sugars as a sole source of carbon. Hence it was concluded that in anabolic processes we were dealing with reactions of a very special kind unknown in ordinary chemistry, so-called involuntary reactions, in which the free energy of the system increases. This was deemed possible because these reactions acquire energy from the simultaneously occurring catabolic processes in which the free energy decreases, and this to such an extent that the free energy of the complex system as a whole decreases as well.

It is easily understood that gradually much opposition has developed against this conception of an energetic coupling of two chemical reactions which do not have any material link in common. It is, therefore, most rejoicing that in the last ten years considerations have been

put forward which seem suitable to free biochemistry from this special vitalistic aspect.

I have already remarked that the greater part of the step-reactions in the catabolic breakdown of carbohydrates are of the equilibrium type. This implies that the addition of many of the intermediate products to the cell must automatically lead to a partial reversal of the breakdown process, *i.e.*, to the synthesis of the carbohydrate. The realisation of this situation made Cori succeed in the long-pursued enzymatic synthesis of glycogen and starch starting from glucose-1-phosphate or Cori-ester. Perhaps still more spectacular has been the successful solution found by Doudoroff and his co-workers for the problem of the synthesis of saccharose, a problem which generations of organic chemists have been unable to solve. With the aid of a phosphorylase preparation obtained from some common bacterium saccharose was readily produced from a mixture of Cori-ester and fructose.

Now these examples of the successful demonstration of the formation of typical assimilatory products like glycogen and saccharose may perhaps not be so very impressive, because of the relative complexity of the substrate – Cori-ester – from which these syntheses start. But there is no doubt that by applying the necessary biocatalysts they could as successfully be accomplished when starting with various much simpler compounds, such as for instance phosphopyruvate.

Since, however, in these cases the potentials of substrates and synthetic products are very much on the same level they do not represent clear cases of energy transfer necessary for the formation of anabolic products of high potential as referred to earlier.

Now it is very satisfactory that also in the elucidation of the mechanism of such anabolic reactions important advances have been made. These advances are for a great deal due to three scientists well-known in Copenhagen so that it will suffice to give only a very brief review of their findings. The development of our insight into this matter has started with Lundsgaard's discovery of the anaerobic phosphocreatine breakdown during a lactic acid muscle contraction, and the coupling of the resynthesis of this compound with lactic acid formation in glycolysis. A second well-defined instance of the coupling of an oxidation with phosphate uptake was discovered by Meyerhof and his co-workers in the oxidation of phosphotriose as a step-reaction in carbohydrate breakdown. They showed that this oxidation led to the formation of

phosphoglycerolphosphate (diphosphoglyceric acid). The important aspect of this observation is that this oxidation is reversible while the analogous oxidation of the phosphotriose without phosphate is strongly exergonic. We may conclude, therefore, that the diphosphoglyceric acid, owing to the energy-rich acylphosphate bond acts as a storage for the potential energy of the oxidation reaction. Both Lipmann and Kalckar have in recent years discovered several other labile compounds with such energy-rich phosphate bonds, and have dealt in theoretically very important studies with their remarkable properties. There seems to be little doubt that these highly reactive compounds with high energy potential which are formed in catabolic processes act as a starting point for synthetical processes which then again consist of chains of ordinary step-reactions, *i.e.*, reactions which proceed with a decrease of free energy.

A striking example in favour of this view may not pass unmentioned. I have already referred to the remarkable metabolism of the so-called chemo-autotrophic bacteria who are able to build up their cell constituents from carbon dioxide owing to the energy derived from inorganic oxidation processes. Until shortly the nature of this energy transfer was still wholly obscure. Vogler, Umbreit and Le Page have now given convincing proof that for the sulphur oxidizing bacterium *Thiobacillus thiooxidans* the formation of organic phosphate compounds provides a material link between catabolism and anabolism. The first advance made by these investigators was the demonstration of the possibility to separate both processes in time. A suspension of the bacteria supplied with a small quantity of sulphur in the absence of carbon dioxide was found to consume oxygen rapidly, and if then after some time carbon dioxide was admitted a rapid uptake of this gas was observed. But the most remarkable fact was that the oxidation of the sulphur proved to be accompanied by the uptake of inorganic phosphate from the medium, whilst in the phase of carbon dioxide assimilation the phosphate was again released. Finally it could be shown that the cells contained several organic phosphate compounds well-known as metabolites in heterotrophic organisms. The intermediate organic phosphate compound which evidently acts as a material link between the energy yielding oxidation and the energy demanding synthesis is to all probability adenosine-3-triphosphate a compound closely related to adenosine-5-triphosphate, a regularly occurring inter-

mediate in yeast and muscle metabolism, and which is one of the best-known examples of a compound with an energy-rich phosphate bond.

Here again we are confronted with a most remarkable unity in metabolism of micro-organisms at first sight so widely separated. It is clear that these important results mean a very real contribution to the removal of the contrast between the autotrophic and the heterotrophic mode of life.

It is impossible to discuss thirty years development of our knowledge of microbiological metabolism without giving some special attention to the change in our attitude towards the rôle of carbon dioxide in this metabolism. We can state that until ten years ago the current views were fully governed by the classical picture of the cycle of carbon in nature. According to this picture carbon dioxide had to be considered as the endproduct of respiration in living animals and plants and of their decay after death under the influence of bacteria and fungi. The carbon dioxide once formed was unassailable by all heterotrophic organisms, and only entered into the second half of the cycle owing to the special action of the green plants in which the radiant energy of the sun managed to bring about the reduction ultimately leading to the formation of the various organic constituents of the plant.

Now I shall not enter into details regarding the various observations which are responsible for a considerable change of these views. To begin with I briefly refer to the fact that the discovery of the chemotrophic bacteria already made exception to the rule that the conversion of carbon dioxide to organic compounds could only proceed with the aid of light energy. But besides this exception of a special nature gradually several indications were obtained which seemed to contradict the idea that in the colourless heterotrophic organisms carbon dioxide is only an indifferent endproduct of metabolism. A fast increasing number of instances were recorded all pointing to the fact that small amounts of carbon dioxide are indispensable to the initiation of growth of a great variety of heterotrophic micro-organisms, and that growth may be either completely suppressed or at any rate considerably retarded by a more or less successful removal of carbon dioxide from the culture. This seemed to indicate that the micro-organisms in question in some way or other managed to utilize carbon dioxide for the synthesis of essential cell constituents.

However, apart from the disregarded observation made by Söhngen already in 1905 that methane bacteria are able to convert a mixture of carbon dioxide and hydrogen into methane, it lasted till 1936 before experimental proof for the utilization of carbon dioxide in special cases of heterotrophic metabolism was given. In that year Woods showed that *Bacterium coli* was able to produce formic acid out of carbon dioxide and hydrogen, whilst Barker succeeded in bringing convincing proof that also in the fermentation of organic compounds by methane bacteria the methane always originated from a reduction of carbon dioxide. Now these conversions of carbon dioxide into other compounds with one C-atom were undoubtedly interesting but they did not throw any light on the problem of the indispensability of carbon dioxide for cell synthesis and growth. For this reason it was of great importance that Wood and Werkman, also in 1936, showed that in the fermentation of glycerol the strictly heterotrophic propionic acid bacteria take up carbon dioxide from the medium, and that for every mole of carbon dioxide reduced approximately equivalent quantities of succinic acid with its 4 carbon atoms are formed. This result may be deemed to be of far-reaching significance, since it strongly suggests that here synthesis of a C₄-compound out of a C₃-compound and carbon dioxide has taken place, which would imply the formation of a new bond between carbon atoms. It is easily understood that this experimental result together with the increasing evidence for the indispensability of carbon dioxide for heterotrophic organisms in general made several investigators surmise that the uptake of carbon dioxide into organic compounds under formation of new carbon-carbon bonds would be a reaction of more or less common occurrence in heterotrophic cells.

In 1939, when lecturing on this subject in Helsinki, I have expressed this view with the following words: 'At present a lecturer still must be prepared to meet with energetic protests when he contends that the cattle at pasture, or even his distinguished audience assimilates carbon dioxide. Nevertheless, the temptation to such an assertion is already there!' Now seven years later the statement in question is no longer open to scepticism, and many of my auditors will think it to be quite commonplace: the synthetic utilisation of carbon dioxide by heterotrophic cells has been indubitably proved for representatives of very much diverging classes of organisms, such as bacteria, yeasts, fungi, protozoa and higher animals.

One might rightly ask for the reasons which are responsible for the fact that such a far-reaching discovery which amongst others noticeably affects our views on the carbon cycle in nature has been made so late in the development of our science. Yet this is easily understood, if one realizes that in all these organisms metabolism is characterized by a *net production* of carbon dioxide thus rendering it virtually impossible to detect any simultaneous reassimilation of carbon dioxide with the usual chemical or manometrical techniques. It is only the application of a new and powerful tool to the problem, namely the use of 'tracer' or 'labelled' elements which has made this progress possible.

On the principle of this method which even in the short time of its application has revolutionized several aspects of metabolism I need not digress, since it is again in Copenhagen that it was first introduced into biochemistry by von Hevesy. The remarkable results obtained by this scientist and his followers on introducing compounds containing radioactive phosphorus into metabolic investigation are universally known. But the importance of these studies have perhaps even been surpassed when Schoenheimer and Rittenberg applied the same principle in their researches on nitrogen metabolism by making use of nitrogen compounds containing the stable heavy N^{15} isotope. It now has been the application of radioactive C^{11} carbon by Ruben, Kamen and their co-workers, and of the stable heavy C^{13} isotope by Wood, Werkman and collaborators that has yielded the incontestable proof for the assimilation of carbon dioxide by heterotrophic cells.

However, it should not be inferred that the purport of the recent investigations with the carbon isotopes is restricted to the mere establishment of this carbon dioxide assimilation.

In the first place it has been possible to identify in some instances the primary reactions in which the carbon dioxide is fixed. Thanks to the use of the labelled carbon there is nowadays no longer any doubt that *Bacterium coli* contains enzyme-systems capable of bringing about the carboxylation of pyruvic acid leading to the formation of oxalacetic acid which acid then is converted into succinic, lactic and acetic acids. It was further proved that in accordance with expectation in all these products the labelled carbon was located in the carboxyl group. But more recent studies by Lipmann and Werkman have shown that carboxylation of pyruvic acid is not the only way in which *Bacterium coli* can fix carbon dioxide. The second way is especially

interesting because it clearly represents another successful intervention of a compound with an energy-rich phosphate bond. We owe to Lipmann the important observation that the conversion of pyruvic acid by bacterial systems into acetic and formic acid is not, as had long been accepted, a reaction in which water takes part, but a phosphorolysis, in so far that the primary product besides the formic acid is not acetic acid but acetylphosphate. By adding formic acid with labelled carbon to the medium it has been definitely proved that owing to the high potential of the acetylphosphate this primary reaction is reversible, and since *Bacterium coli* is also able to form formic acid out of carbon dioxide and hydrogen it is now quite certain that carbon dioxide can also enter the metabolism via a synthesis of pyruvic acid.

I cannot dismiss the subject of heterotrophic carbon dioxide assimilation without leaving for a moment the microbiological field, and asking your attention for analogous studies made in animal metabolism. Here in some cases it has been possible to trace the path which the labelled carbon follows in the intricate web of metabolic processes. Amongst others this has led to the startling observation that part of the labelled carbon injected intraperitoneally in the form of a sodium bicarbonate solution into fasted rats can within a short time at least partly be found back in the liver glycogen. And Wood and his coworkers have even succeeded in bringing experimental proof that this labelled carbon is located in the positions 3 and 4 of the glucose molecules which are the building stones of the glycogen. This result is so gratifying because it is exactly what might be expected on the basis of the current reaction schemes in the supposition that glycogen synthesis proceeds as the reversal of glycolysis.

Finally it should be observed that in the last three years the use of the stable carbon isotope has found application far beyond the problem of carbon dioxide assimilation. Werkman has succeeded in preparing various acids, such as acetic, lactic, propionic and butyric acids, containing heavy carbon atoms either in the carboxyl groups, or also in other locations in the molecules. By adding such compounds to the usual fermentation media of various types of bacteria they have been able to trace conversions of these molecules which conversions had at least partly a quite unexpected character.

For the moment the number of these observations is still relatively small, but they suffice to show that until shortly the metabolic capac-

ities of the microbial cell have been badly underrated. It now has become increasingly clear that in the metabolizing cell we are dealing with a system of equilibrium reactions in which a continuous interplay between cell constituents, intermediates and so-called endproducts takes place.

This newly gained insight strongly contrasts with the current conception of the preceding period in which fermentation processes, and catabolism in general, were considered more or less as cascades unchained by a more or less stable system of catalysts, and in which a suitable substrate via labile intermediates was transformed into a number of stable endproducts fixed for every type of cell.

It is evident that the introduction of isotopes has brought the study of intermediate metabolism on a level much higher than it had reached before. For it cannot be denied that until recently investigators could not go much farther than to demonstrate that a certain type of cell contained enzyme systems which are capable of bringing about some intermediate step-reaction. But this did not imply that under a given set of metabolic conditions this particular step-reaction does indeed take place. As a matter of fact there have been moments that microbiologists have been struggling with a surplus of intermediate products!

For these reasons it has to be admitted that the drawing up of a scheme for the internal mechanism of a biochemical process on the basis of intermediate products isolated under special conditions has always been afflicted with elements of a speculative character.

If we contrast this situation with the degree of certainty obtainable by the application of labelled atoms which, if properly examined, give straightforward answers to many questions, it is not too dangerous to predict that in the future studies on the intermediate metabolism will almost completely be based on the use of isotopes. Moreover, it does not seem excluded at all that these studies will alter profoundly several of our current views.

There are, however, some considerations which tend to moderate these hopeful expectations, and that is in the first place the at present still very restricted availability of many isotopes, more especially of the carbon isotopes.

In order to apply the radioactive C^{11} carbon successfully it is necessary to work in the immediate neighbourhood of a powerful cyclo-

tron, since the half life time of this isotope is only 21 minutes, restricting its applicability to experiments of a duration of about two hours.

In this respect the stable C^{13} is, of course, quite preferable, but alas the isolation of this isotope obviously still asks for a very cumbersome procedure. This may be inferred from the fact that both Werkman and Wood and their schools have been supplied with C^{13} by the physicist of Minneapolis Professor Nier and that this scientist recently stated that he holds the world record for C^{13} -production amounting to 5 grams annually! And when reading the hopeful statements in scientific journals that this year the Sun Oil Corporation of Philadelphia will start the commercial production of C^{13} it is not generally realized that the annual production by this firm is estimated to be 500 grams! With these facts before our eyes it will be wise to be not too optimistic regarding a world-wide application of carbon isotopes in a near future.

In addition also the following should not be neglected. Publications on metabolic studies with the aid of the stable carbon isotope are as a rule very attractive for the reader, the results reported usually being simple and clear-cut. There is, however, a great danger that one will be insufficiently impressed by the enormous amount of experimental work which has been required in order to obtain these results. In many cases it has been necessary to search a great number of compounds on a possible excess content of labelled carbon, with only a restricted number of positive results. Especially when an attempt is made to arrive at a complete balance of labelled carbon added and recovered many very careful analyses with the mass-spectrometer are indispensable. And if finally it becomes necessary to fix the location of the heavy carbon atoms in the molecule of a metabolic product, various purely chemical degradation reactions of this product have to be studied before the mass-spectrometer can again give the desired answer. To this should be added the necessity of carefully recovering in all this work the precious isotope!

All this asks for a more or less specialized staff of workers and, therefore, tends to limit the immediate universal introduction of the isotope method in the study of metabolism. It is, therefore most welcome that in the last years in the United States quite another line of attack in the study of metabolic processes has been discovered which seems to be full of promise and to which I shall return later on.

A survey of thirty years progress in microbiological metabolism would be very incomplete, if not due attention was given to the enormous amount of very successful work which has been performed on the subject of growth factors, leading to the discovery of the rather common occurrence amongst micro-organisms of a phenomenon that later has been termed auxo-heterotrophy by Schopfer. As is well-known the first observations regarding this phenomenon date back to 1900 when Wildiers demonstrated that a certain strain of yeast would only proliferate in a medium containing sugar and the necessary quantities of inorganic nutrilites, if a small quantity of yeast extract or some other vegetable extract was added to the medium. For the growth-promoting principle present in these extracts Wildiers coined the term bios. The development in this field is too generally known to be in need of further documentation, yet it seems worth while to devote a few remarks to the general aspects which have resulted from these studies.

In the first place it has been definitely proved that the various growth factors which have appeared to be essential – often in surprisingly small quantities – for the development of certain micro-organisms are nearly all known to act also as accessory food factors in animal metabolism. Wildiers' bios has been shown to be composed of various factors which could be identified as biotin, thiamine, pyridoxine, inositol, pantothenic acid, nicotinic acid and p.aminobenzoic acid, and for all these compounds it has now definitely been established that they are indispensable food constituents for special types of test animals, on the understanding that their absence in the food either inhibits growth or leads to certain other defects.

A second important viewpoint is that already much light has been thrown on the way in which these mysterious growth factors exert their beneficial action on the cell, apparently many of them, if not all, are essential components of certain enzyme systems. So for instance thiamine has been found to be the active group of the cocarboxylase, the enzyme which decarboxylates pyruvic acid, nicotinic acid is the active group of the dehydrogenase-system, riboflavin is part of one of the respiratory enzymes, and recently it has been proved that a pyridoxine derivative is the active principle in the decarboxylation of amino acids.

An at first sight disturbing factor is that a phylogenetic study of the distribution of the property of auxo-heterotrophy has learnt that on

the one hand next to species that are in need of growth factors there are closely related species which can do perfectly without, and that on the other hand the need for a special growth factor is met with in very divergent classes of plants and animals. However, it has been clearly shown that the independence of the so-called auxo-autotrophic organisms of the presence of growth factors in their food is not due to the fact that they can do without these factors, but that they are able to synthesize the compounds in question. This implies that the auxo-heterotrophic cells have obviously lost these synthetic capacities, and such a loss has apparently occurred at very different stages in the course of evolution.

Following Schopfer who has made so many important contributions in this field, the situation may be sketched as follows.

One gets the impression that at all levels of evolution one has to do with a living substance that is essentially the same. But during the trend of evolution this living substance has started to lose its independence, the losses in synthetic ability increasing with increasing morphological complexity and increasing adaptation to a heterotrophic life. Yet the main lines of metabolism have been maintained, the ultimate requirements have remained unchanged. The differences between the various physiological groups of organisms are not to be found in differences in the fundamental constitution of their living substance, but just in the different ways in which the latter originates. Whether an organism itself synthesizes the active groups of its enzyme systems, or has to find these groups ready-made in its medium, is only of secondary importance: the final result being the same in both cases.

We may conclude from all this that the study of microbial growth factors in close contact with that of vitamins have also led to an impressive manifestation of a fundamental unity of metabolism in the whole kingdom of life.

On looking back on so many advances in our insight into the principles underlying microbial life one may ask whether future research will not necessarily be restricted to an extension and a consolidation of these findings. It may appear that prospects for further developments of a more essential character are far from bright.

In reality there is already ample evidence that future generations

of microbiologists will find vast areas of their science still awaiting cultivation.

Until now I have only been dealing with observations made on micro-organisms as they have been isolated from their natural habitat, and the constancy of their properties during these observations has been a postulate for the discussion of the results obtained. This means that one of the most fundamental characters of every form of life, *viz.*, its variability, has been left out of consideration.

Now it is a common experience of every microbiologist that not rarely a continued study of some micro-organism leads to the conclusion that variations have occurred.

Fortunately in discussing the possibilities inhaerent in microbial variations it is not necessary to have recourse to the still difficultly interpretable results obtained with bacteria. There are other groups of micro-organisms in which because of the occurrence of sexual reproduction the nature of the variation phenomena is open to a detailed analysis.

Amongst the various studies made in this field the series of investigations in recent years carried out by Beadle and his collaborators at Stanford University on the biochemical genetics of the mould *Neurospora crassa* may be deemed to be outstanding, and there are even scientists who expect that this mould will in the course of time supersede *Drosophila* from its central position in genetics. How this may be, the said investigations are in any case apt to give a clear idea of the importance which induced mutations may well obtain in the future development of microbiology. It is for this reason that it seems worth while to spare some moments for the discussion of some of the results obtained.

Neurospora crassa, commonly known as red bread mould, is heterothallic, that is, exists in two morphologically identical but physiologically different sexes. Each of these is haploid and by itself reproduces only vegetatively by mycelial growth or through the mediation of asexual spores. If mycelia of the two sexes are grown together on a suitable medium hyphal fusions accompanied by nuclear fusions occur and out of the diploid zygotes ultimately asci containing 8 linearly arranged ascospores are formed. Owing to the meiosis occurring in the ascus preceding the ascospore formation the ascospores are again haploid. If the parent strains differ in the alleles of a single gene, the eight

ascospores of an ascus are of two kinds, four like one parent and four like the other. It is evident that this situation provides a means to check whether a property characteristic for some induced mutant is dependent on the mutation of a single gene.

Beadle and his collaborators have now succeeded in obtaining a large number of biochemical mutants by treating asexual spores with X-rays or ultraviolet light, making crosses with strains of the opposite sex, and then establishing genetically homogeneous, single ascospore strains. Special attention has now been given to the question whether these strains differed in their growth requirements from the original, wild-type strain. For the latter it had previously been established that it grew quite satisfactorily in a medium containing some sugar, the necessary inorganic salts – nitrate as a source of nitrogen – and biotin as the only indispensable growth factor. For many of the mutants obtained it was found that they no longer developed in this minimal medium, but that they did so perfectly well if some extract containing the various amino acids and B-vitamins was added. This result can only be understood on the basis of the assumption that the ability of the wild-type strain to synthesize one or more of the essential amino acids and B-vitamins was lost in the mutation act. By composing a series of media, in each of which a single amino acid or one of the growth factors had been added to the minimal medium, and observing in which case growth occurred after inoculation with the mutant strain, it was, of course, possible to establish for this particular strain the specific loss in synthetic ability. By this procedure it could be shown that amongst the numerous mutants obtained there were strains which differed from the wild-type strain by loss of the ability to synthesize one of the following compounds: thiamine, pyridoxine, p-aminobenzoic acid, pantothenic acid, inositol, nicotinic acid, choline, arginine, lysine, leucine, valine, methionine, tryptophan, proline and threonine. Moreover, the genetical analysis showed that each of these strains differed from the wild-type by a single gene, the normal allele of which is evidently essential to the biosynthesis of the compound in question.

A further biochemical investigation of some of these mutant strains has led to some extremely interesting results. It is clear that the biosynthesis of each of the compounds mentioned depends on a long chain of individual step-reactions, and that the blocking of any of these step-

reactions will suffice to prevent the synthesis of the compound in question. Since there were already several indications that each particular step-reaction is determined by one gene, it might be expected that mutants failing in the synthesis of one and the same essential compound may differ mutually in the nature of the step-reaction blocked.

This expectation has now been amply confirmed in an investigation of a great number of mutants deficient in their ability to synthesize the amino acid arginine. Amongst these not less than 7 different types have been recognized. Independent biochemical evidence had made it probable that the synthesis of arginine would proceed in this way that first a still unknown series of reactions would lead to the formation of ornithine which then was converted into citrulline which then in a final reaction would yield arginine. It was therefore tested in how far either ornithine or citrulline could meet the growth requirements of the various 'arginine-deficient' strains. It was then found that for one mutant these compounds could not be used as a substitute for arginine itself. Evidently here the final step-reaction had been blocked. For a second type of mutants citrulline was acceptable, but ornithine not, indicating a blocking of the ornithine-citrulline step-reaction. For the remaining strains both ornithine and citrulline were suitable substitutes for arginine which must imply that one of the step-reactions in the long chain leading to ornithine synthesis had been blocked. Since these strains differed mutually, it was obvious that here too in each of the strains different step-reactions were inactivated.

In the first place these results form a strong argument for the one-gene-one-enzyme concept which in slightly different terms was already so passionately advocated by my famous predecessor Beijerinck as long ago as 1917. But it is also clear that the production and investigation of biochemical mutants form a powerful tool for the analysis of anabolic processes in the microbial cell. It will be clear that in many cases it will be possible to submit the correctness of assumptions regarding the way in which various cell constituents are synthesized to an experimental test by studying the behaviour of special mutants towards supposed intermediate products.

It is in this way that Tatum, Bonner and Beadle have made it very probable that the ultimate step in tryptophan synthesis is the condensation of indole and serine, and that moreover anthranilic acid is an

immediate precursor of indole. The latter conclusion is based on the fact that if one of the tryptophan-deficient mutants is grown in the presence of a minimal amount of tryptophan the mutant accumulates anthranilic acid and excretes it in the medium from which it has been isolated in the crystalline state.

Here we have the interesting example of a biochemical mutant forming a metabolic product quite unknown for the original type. Is it not tempting to suggest in this connexion that the superiority in penicillin production of several mutant strains of *Penicillium notatum* obtained by radiation with X-rays or ultraviolet light, or by bombardment with neutrons, is due to the inactivation of a gene determining an enzyme by which in the wild strains penicillin is converted into some other cell constituent?

However this may be, it is not doubtful that the microbiologist of the future will be able to produce microbes with potencies differing markedly from those known up to this time. And the case of penicillin and other antibiotics is there to testify that the production of even minute quantities of metabolites with new properties may become of far-reaching importance for mankind.

Our conclusion then must be that the coming generations of microbiologists, far from being pitiable, will experience joys both from an increased insight into the mysteries of microbial life and from an increased mastery of its manifestations. And the industrial microbiologist will add one key industry to those already existing: the industry in which microbes with desired qualities will be fabricated!

The lecturer is, of course, aware of the danger inherent in such prophecies, and for this reason it seems high time to return to reality. And then I think I may not conclude without devoting a few special words to the progress made regarding our knowledge of that group of micro-organisms which was nearer to Emil Christian Hansen's heart than any others: the yeasts. It is in this domain that his name is rightly perpetuated in the generic names *Hansenula* and *Hanseniaspora*.

If at this moment he could have been with us, what would he say? Would he be satisfied with the way in which we his successors have taken care of the heritage he left to us?

After all that I have learnt about the character of this great scientist I do not expect that he would be exuberant in his praise. It is well-

known that he feared that such praise might lead to relaxation, instead of acting as a stimulant to further efforts.

But, nevertheless, I venture to think that in his heart a feeling of satisfaction would not be lacking. In the first place this seems to be most probable, when we remind ourselves of the fact that during his life-time Hansen had to devote part of his energy to the defence of the view that the yeasts which he brought together in the genus *Saccharomyces* have to be considered as independent forms of life, and not, as was repeatedly suggested, are mere stages in the life cycle of higher fungi. Seen in this light Hansen would have relished in the present situation in which about 100 different ascosporeforming yeast species, divided over 15 genera, are generally accepted as independent organisms in the botanical world. Moreover, he might have rejoiced at seeing that attempts to deal in a somewhat comprehensive way with the systematics of yeasts, of which systematics the foundations were laid by him, have necessitated the publication of three more or less stately volumes.

In view of his great devotion to the industrial applications of yeasts, Hansen would also have been interested to see that the organisms which are undoubtedly responsible for the particular qualities of special types of English beers continue to receive the attention of scientists of the small nations. Herewith I refer to the remarkable group of yeasts first discovered and described by your fellow-countryman Hjelte Claussen, and to which the name of *Brettanomyces* was given by him. Later on they have once more been most thoroughly studied in Copenhagen by Schiønning, and I am glad to be able to add that my collaborator Custers of late could prove that British conservatism has continued to safeguard during another 40 years the hospitality offered by British breweries to a group of micro-organisms, the natural occurrence of which is still wrapped in mystery.

There also seems no doubt that it would be a source of true satisfaction to Hansen to see that nowadays the industrial application of yeasts is no longer restricted to his two favourite species: *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*, but that for the production of food yeast, fat or vitamins several other species have been, or are likely to be introduced into industry in a near future.

But all these minor joys which Hansen might experience, if he was able to inspect his old kingdom, would appear to him as mere trifles

after having given due attention to what had been achieved in his own beloved Carlsberg Laboratory. There he would be confronted with the situation that the president of the Emil Christian Hansen 'Fond' had revolutionized his science and opened wide prospects for future developments by applying the mighty tool of genetics to the study of the yeasts. Undoubtedly the fact that it has been possible to produce consciously new strains with interesting properties by hybridisation would have quite especially appealed to the man who already in 1891 published the first observations, at that time not well interpretable, on ascospore copulation in *Saccharomyces cerevisiae*. The idea that the phenomenon of variability in *Saccharomycetes*, to which he already half a century ago had devoted so much attention, has at last successfully been submitted to a careful analysis, and gradually is giving up its secrets, would be another reason for rejoicings.

The realisation that just like in his own days the Carlsberg Laboratory is still the world's leading centre in yeast science would make even Hansen's stern character break with its principles, and we may presume that he would be unable to withhold words of praise and satisfaction.

HOMO MILITANS

WHEN, in 1901, the 'Hollandsche Maatschappij der Wetenschappen' decided henceforth to include a special lecture on some scientific subject in the programme of its general meetings, it was also stipulated that this should be allocated in turn to different branches of science. This implies that the member whose privilege it is to deliver one of the annual lectures faces a grave responsibility with respect to the particular branch he represents. This is so because the manner in which he fulfills his task cannot fail to influence the appreciation of his specialty on the part of the directors and fellow members of the society. As a microbiologist it is therefore my duty to evoke in my audience, largely composed of laymen in the field, an interest in our knowledge of the microbial world. Hence it has seemed to me that I should attempt to make my audience 'microbe-minded', at least for the time span that will elapse till our next annual meeting, when new impressions are apt to displace the present ones. Consequently I decided to elaborate the thesis that, even though most of you have thus far benefited from neglecting the microbial world, the microbes themselves are not always prepared to neglect mankind. In other words, it seemed an attractive task to discuss briefly the man-microbe relationship.

On further consideration I soon realized, however, that within the prescribed time limit it is not feasible to review this relationship from all angles, and that I would have to restrict myself to a few aspects. I might, for example, have discussed the microbes as benefactors of mankind, and depicted the disconsolate horrors of human life on a microbe-less world. But rather than doing this I have decided to direct your attention to the far less numerous microbes inimical to man. On the one hand it are particularly these microbes that have left their direct imprint on the history of mankind; on the other hand this topic seemed to offer an opportunity to elicit your interest in man's incessant fight against his assailants from among the realm of invisible life.

It was during this phase of my deliberations that I decided to use '*Homo militans*' as the title of my lecture. Soon afterwards I learned

that a few years ago a book had been published with the self-same title. After acquainting myself with its contents, I determined to deal with my subject in a somewhat larger framework. In the book, published shortly before our country too became involved in the war, the psychiatrist Meerloo has tried to analyse the human factors that cause wars. In particular I was struck by the passage:

Is 'not all mutual conflict abolished by a still greater external danger, in the case of people as well as of countries? Were Mars to engage in a war against the Earth, the United States of the World would be an accomplished fact.'

Now it seems to me that as yet there is little prospect of an inter-planetary war, notwithstanding the fact that the recent developments in radar technique have permitted the establishment of an initial contact with the moon, and that the first terrestrial rockets may soon reach it. If, therefore, cooperation of mankind would have to await such an interplanetary war, the prospects would be very dim. But in this connexion the question arises whether the dangers to which mankind as a whole is exposed must needs be of extraterrestrial origin, which immediately leads to the second question whether man is actually the undisputed ruler of the earth he generally fancies himself to be. And if the latter question should have to be answered in the negative, is it not then indicated, nay, imperative that mankind unite in order to stave off the threatening dangers?

Viewed in this light it seems advantageous to stir up the recognition that mankind is indeed exposed to pressing dangers. We must clearly realize that, apart from man, an immense number of other forms of life are engaged in the struggle for existence on earth. And wherever life encounters life there will be strife; contest for space, and, especially, for food. It is altogether insufficiently appreciated that man, too, is fully implicated in this struggle, and that the issue is far less certain than the large majority of the ignorant among us appears tacitly to assume. Hence I shall discuss a few episodes of this struggle which man has had to fight from the moment of his appearance on earth. Obviously the emphasis will be on the fight against invisible life, *i.e.*, the microbial world; but it must be pointed out that more highly organized beings also constitute a real threat.

There is but one enemy of *Homo sapiens*, and probably the most frightening of all, whom I shall deliberately disregard; this is his

brother, *Homo ignorans*. Whosoever wishes to become deeply disturbed by the manner in which, through ignorance and thoughtlessness, man himself is engaged in undermining his terrestrial existence should read the recent book by Fairfield Osborne, 'Our Plundered Planet'. In this treatise, dedicated 'to all who care about tomorrow', the famed President of the New York Zoological Society demonstrates how man, in consequence of the alarmingly rapid population increase, has resorted more and more to a rapacious exploitation, and thus has gradually become a geological force that even now is rapidly changing the once flourishing appearance of our planet into something akin to the desultory landscape of the moon.

In this connexion it is instructive sharply to focus on the data concerning the growth of the earth's population. It is estimated that in 1630 the total number of humans amounted to 400 millions; that this number was doubled in 1830, and again in 1900. In 1940 it was certainly in excess of 2,000 millions, and, if the present rate of increase of 1 per cent annually is maintained, it will have reached 4,000 millions by about 2010!

One might be inclined to conclude from these figures that man has managed pretty well in his struggle with non-human life. But one would do well to realize that it are exactly the problems incident to this enormous increase in human population that will increasingly tax the activity of *Homo militans*.

In beginning a survey of some enemies of mankind one may be apt to think primarily of animals, besides the invisible single-celled organisms. But those who are familiar with life in tropical jungles will realize that products of human culture may also be subject to continuous attack by higher plants.

Many whose privilege it has been to sail, as I did, from Holland to Insulinde prior to 1940 will cherish the powerful primary impression created by the grand virginity of its mighty tropical growth. During the voyage from Padang to Tandjong Priok, at that time still occupying two days, nothing struck me as much as the dense and luxurious green vegetation that covers Sumatra's West coast and the smaller islands we passed. Not a trace of human habitation was to be observed; and even though rationally one had to infer that here and there man must occasionally have penetrated, it was evident that he

had not succeeded in leaving his imprint on the landscape. In view of the statements of our airplane pilots, who complain of the monotony of the scenery when flying across Sumatra, and who use in this context the pithy term 'kale', this situation appears not to have changed. On reaching the densely populated island of Java this initial impression is, however, rapidly modified. Here the physiognomy of the landscape is largely determined by the cities, the dispersed, yet practically omnipresent farm buildings, the extensive sawahs of the natives, and the large agricultural areas under occidental management.

Nevertheless, it soon dawned upon me not merely that this human intrusion into the tropics had been accomplished at the expense of a great deal of energy, but also that it could only be sustained through persistent and great effort. This was strikingly brought home to me by the ruins I beheld during an excursion in the Bantam district; they had become engulfed in plant growth, and only the barely discernable inscriptions 'V.O.C.' still indicated that here our ancestors had built and inhabited their fortifications. It was obvious that not much more time would elapse before the last traces of human influence would here be erased.

However, this simple experience is not likely to impress my audience. Let me therefore request your attention for the description by one who has expressed essentially similar impressions in a masterly fashion. It is Pierre Loti who, in 'Un pèlerin d'Angkor', has reported on his voyage, in 1901, to Angkor in the interior of Indo-China, where enormous ruins, since then restored by the French, bear witness to the imposing and age-old domain of the Khmers. Here a Brahmin civilization was established which built a vast number of temples, richly ornamented with sculpture; a civilization that in turn was superseded and ennobled by a Buddhist culture. Loti writes:

'Il semble que, sous le bouddhisme, la ville d'Angkor connut l'apogée de sa gloire. Mais l'histoire de son rapide et mystérieux déclin n'a pas été écrite, et la forêt envahissante en garde le secret. Le petit Cambodge actuel, conservateur de rites compliqués au sens perdu, est un dernier débris de ce vaste empire des Khmers, qui depuis plus de cinq cents ans a fini de s'éteindre sous le silence des arbres et des mousses . . .'

Loti provides a vivid description of the long voyage he had to undertake before at last confronting the heavily overgrown walls of ancient

Angkor-Thom. He entered through a sombre gateway, covered with monstrous Brahma statues; but even here the primeval forest, with its population of snakes and monkeys, persisted, while in its midst the innumerable vestiges of architecture and sculpture, reminiscent of the ancient grandeur, had become virtually amalgamated with the vegetation. Let me quote Loti:

‘Car il y a un entêtement de destruction même chez les plantes. Le Prince de la Mort, que les Brahmes appellent Shiva, celui qui a suscité à chaque bête l’ennemi spécial qui la mange, à chaque créature ses microbes rongeurs, semble avoir prévu, depuis la nuit des origines, que les hommes tenteraient de se prolonger un peu en construisant des choses durables; alors, pour anéantir leur oeuvre, il a imaginé, entre mille autres agents destructeurs, les parietaires, et surtout ce “figuier des ruines” auquel rien ne résiste.

‘C’est le “figuier des ruines” qui règne aujourd’hui en maître sur Angkor. Au-dessus des palais, au-dessus des temples qu’il a patiemment désagrégés, partout il déploie en triomphe son pâle branchage lisse, aux mouchetures de serpent, et son large dôme de feuilles.’

To be sure, many factors other than tropical plant growth have cooperated in the dissolution of one of the grandest expressions of human culture. Nevertheless, here one finds the most striking demonstration of the transitoriness of human endeavour which, even in its most durable forms, can be maintained only by perpetual struggle against non-human forms of life.

In passing it may be remarked that the preceding passages also serve to cast a new light on the proud device of the House of Orange, ‘Je Maintiendrai’; in its opposition to *παντα ἔει* it is perhaps the loftiest of watchwords of all times.

It is almost superfluous to indicate that the manifestations of human culture are subject to attack by living agencies other than the tropical vegetation mentioned above in connexion with the extreme case of the annihilation of mighty structures. Most of these expressions are after all of a much more fragile kind. One needs but think of paintings, whose organic substructure provides a fully acceptable nutrient substrate for many insects, moulds, and bacteria. It is gratifying that, when the dangers of war were perceived in our country, the responsible authorities paid timely attention to this fact by storing the paintings and tapestries of our museums in underground vaults where,

through the regulation of temperature and humidity, the said enemies could be held in check.

A far more direct threat to mankind lies, however, in the fact that its perpetuation is dependent upon an incessant supply of food which is ultimately provided by agriculture. It goes without saying that our cultivated plants also furnish a basis for the existence of numerous other forms of life, so that both the crops in the field and the harvest products during storage are incessantly subject to attack by non-human organisms. It is impossible to overemphasize the fact that in this respect the dangers have enormously increased during the past century. Owing to the large increase in population an ever expanding part of the earth's surface has been brought under cultivation; and as a result the vegetation has taken on a more and more one-sided character. This implies that pestilences, once they get a foothold in some cultivated crop, may spread in a more or less avalanche-like manner, entailing catastrophic consequences for mankind.

As early as 1915 the American entomologist, Johnston, declared that the next war would be fought by humans against insects. And, although this prophecy has only partially been fulfilled, nobody who has taken cognizance of L. O. Howard's book, 'The Insect Menace' published in 1931, can avoid the conclusion that it is imperative for humanity's High Command to concentrate more than hitherto on the war against insects.

Howard, the long-time Head of the U.S. Department of Agriculture's Bureau of Entomology, first of all points out that palaeontological data leave no doubt that insects have maintained themselves on earth for at least forty million years, and probably much longer, whereas during this epoch many other animal species have disappeared. In contrast, the human population probably extends over not more than 500,000 years, and hence, geologically speaking, is merely in an experimental stage. Besides, qualified experts estimate the total number of insect species now populating the world at 2-10 million, of which the entomologists have described less than a tenth part. Even though as yet the number of species that has attacked man and his produce on a large scale is relatively small, one shall have to reckon with the probability that among the still unknown species many potential enemies of mankind may be hidden which at any moment,

under the influence of changes in environmental conditions, could manifest their presence and potency.

A balanced survey of what the economic entomologist nowadays has wrought to protect mankind falls outside the scope of this lecture, and is also beyond my competence. However, let me illustrate by a single example the range and importance of the work of this variety of *Homo militans*. I have chosen the fight against locusts because this has also shown the need for international cooperation. It is but necessary to recall the biblical description of the 'eighth plague' in Exodus 10 in order to realize that from olden times locust plagues have been dreaded by many peoples as harbingers of famine and misery. For centuries man's only defense has consisted in a hopeless and ineffectual attempt to destroy the insects there where they settled down in swarms. A new element in the war against this plague was introduced in 1921, when Uvarov formulated his phase theory of locust development. This investigator established that the larvae of many locust species frequently begin by developing into the so-called *solitaria* phase, differing both in shape and in behaviour from the swarming insect to such an extent that it was believed to represent a different species. During this phase it causes little damage, and its migration is limited. But when conditions cause a great many of these *solitarias* to aggregate in a small region, they develop into the morphologically distinct *gregaria* phase which is extremely voracious, and shows a strong tendency to mass migration.

International investigations have clearly demonstrated the great significance of this developmental history for the problem of warfare against locusts. Thus it has been established that the swarms of the African locust, *Locusta migratoria migratorioides*, which in 1928-1937 ravaged no less than 10 million square miles in such distant parts as British and French West Africa, the Belgian Congo, Sudan, Eritrea, British East Africa, and Rhodesia, were all initiated by the *solitaria* phase occurring within an area of only ten thousand square miles, in the swamplands of the Niger in the French Sudan.

The various investigations have clearly shown that the rational means to prevent disasters in far removed regions consists in controlling the development of the relatively harmless *solitaria* phase. Obviously, this requires international cooperation. During the Fifth International Locust Conference in Brussels, in 1938, it was therefore recom-

mended that an international service be established, a project that has not yet been activated in consequence of the outbreak of the war. But since 1930 the 'Anti-Locust Research Centre' has been working in London, coordinating the data supplied by numerous countries, and sending out warnings and predictions based on these data.

During the war years the British Government initiated important international research on a smaller scale concerning the fight against the so-called desert locust, whose swarms threatened the food sources of East Africa, the Middle East, and British India. This species breeds in the British Sudan and Eritrea, migrates from here *via* the Dead Sea to Arabia and Northwest India, whence it invades the entire territory surrounding the Persian Gulf. From Arabia it generally threatens the valley of the Euphrates, Syria, Palestine, and the Nile Delta. Closely cooperating teams of English, Egyptian, and Indian investigators have kept Arabia under control, while Russian missions have provided additional support in Iran. I cannot enter into details concerning the manner in which the war against the insect has so far been prosecuted, and merely indicate that trenches, wire obstructions, flame throwers, and airplanes took their place among the means of combat. Even more important is, however, the fact that particularly during the past few years poisoned bait has become dominant owing to the manufacture of certain chemicals, extremely small amounts of which can kill insects whereas they are but little poisonous to higher organisms. This method consists in mixing some foodstuff that attracts locusts, such as moist bran, with 0.5 per cent of crude hexachlorobenzene, containing around 13 per cent of the toxic 'gammexane'. An experienced eradicator may kill five million locusts per acre with only 2.5 kg of poisoned bran, containing only 2 g of the insecticide proper.

Although this topic could be expanded practically indefinitely, I shall refrain from further elaborations, not, however, without just raising the spectre of the calamity that Europe would suffer if a relaxation of human vigilance were to lead to a destruction of our potato crop by the dreaded Colorado beetle which has already been collected in our country in litre quantities.

I may not neglect to remind you of the fact that it is not only insects that threaten our food crops. It is generally known that in 1845 and 1846 Western Europe suffered from famine because the fungus, *Phytophthora infestans*, annihilated a large proportion of the potato har-

vest. That in later years this plague has not made itself felt to the same degree is exclusively due to the powerful defense measures that were devised by the phytopathologists. Even the meteorologist has been recruited for this fight, as many of you will realize from the radio messages sent out during the summer.

Besides insects and fungi mention should be made of a totally different class of living organisms that constitute a threat to our food provision. No doubt you will know that numerous plant diseases have now been shown to be caused by viruses, *i.e.*, contagious entities so small that they pass through filters with pores that retain every particle that can be seen with the aid of even the best microscopes. The remarkable feature of these viruses is that at least some of them have been isolated in a pure form from infected plants, and have been characterized as definite chemical substances, *viz.*, nucleoproteins. Naturally this has raised the question whether these agents may lay claim to the predicate 'living', a notion that is supported by their ability to multiply in the diseased plant. I shall not discuss this problem now, and would rather call your attention to the fact that the phytopathologists are unanimous in their opinion that lately the number of virus diseases encountered among our cultivated plants has greatly increased, and apparently is still increasing. Professor Smit, Rector Magnificus of the Agricultural College, has recently discussed this aspect in a trenchant manner, and elaborated the idea that this increase in viruses should be viewed as nature's reaction against man's abuses of the earth's surface. Behind this army of viruses we may discern the spectre of *Homo ignorans*.

If in this connexion we realize that it is currently necessary to adopt extensive measures in order to keep our plant material free from viruses, a factor on which our present significant export of seed potatoes is based, it will be clear that in future an even heavier and more extensive task will be delegated to *Homo militans* in this particular field.

But non-human forms of life threaten humanity not merely by curtailing the food supply. Numerous living organisms, among which the bacteria and viruses predominate, are potentially able to choose man himself as a nutrient substrate. Too little does the layman realize that the absence of pandemics during the past few decades, and hence the fact that infectious diseases have not made serious inroads on human

life, is exclusively the result of the continuous vigilance of the mutually cooperating health services in the various countries. This international cooperation has found its highest expression in the 'World Health Organization', established by the United Nations in 1946 in continuance of a similar activity of the League of Nations. Especially because present-day man has thus been lulled into sleep with respect to the dangers of infectious diseases it does not seem superfluous to review some examples of the pestilences that have seriously threatened the perpetuation of mankind in the course of time.

In the first place I refer to the succession of bubonic plague epidemics that ravaged Europe and the other parts of the known world during the fourteenth century. In F. Eberson's book, 'The Microbe's Challenge', we find the estimate that 25 million persons died of this disease in Europe alone, and around 60 million in all; this amounts to approximately one quarter of the entire human population at that time. An excellent description of the symptoms of the disease, which unambiguously points to the diagnosis of bubonic plague, we owe to Boccaccio who fled before an outbreak in 1348, and chose voluntary exile in Florence where he wrote his 'Decameron'. At present it may be considered well established that the successive epidemics reached Europe, *via* the Near East and North Africa, from Asia where the disease is endemic. It is interesting to note that even in those days man made some timid attempts at defense. In various harbour cities the idea gained ground that the disease apparently was introduced by way of ships, and in 1383 Marseille issued a decree by which in case of suspicion the ship's crew was forced to remain on board for forty days before permission was given to disembark. It is this decree that even nowadays is one of our indispensable defense measures, and although the time span is no longer adhered to, it is responsible for our use of the word 'quarantine'.

I shall not review the numerous bubonic plague epidemics that scourged Europe in later centuries, though in passing I wish to mention the epidemic of 1663-1668 that also affected our country. In 1663 the number of deaths from the plague in Amsterdam alone amounted to 10,000 out of a total population of 200,000; during the next year the number even increased to 24,000.

Not until 1894 did Kitasato and Yersin independently show that bubonic plague is caused by a bacterium, now designated as *Pasteur-*

ella pestis, while soon afterwards various investigators furnished proof that plague is primarily a disease of rats and other rodents, with fleas acting as the means by which the germs are transmitted from animal to man. Since that time the war against bubonic plague has been primarily a war against rats.

It may here be remarked that plague epidemics, after all, have spontaneously disappeared from Europe, while man has largely remained passive. But then it must be pointed out that in Asiatic countries this disease continues to make victims on a large scale. In British India alone more than 10 million people have died of plague during the period 1898-1918. One may well shudder at the thought of what might have happened in the densely populated island of Java if during the past few decennia the 'Plague Control Service' had less energetically fulfilled its task of rendering the homes of the Javanese population ratproof, and if Otten had not prophylactically treated 94 per cent of the two million inhabitants of the Preanger district with the live vaccine he had prepared.

The pneumonic plague epidemic in Manchuria, characterized by a very high mortality, and in which not the rat but the locally abundant Siberian marmot ('tarbagan'), cherished for its fur, turned out to be the source of the infection, caused a great stir in 1910. A second epidemic, which broke out ten years later, was successfully controlled by the meanwhile established 'North Manchurian Plague Prevention Service', notwithstanding the extremely difficult conditions under which it had to function, thus causing the number of victims to be greatly reduced. It must also be borne in mind that even in this century occasional cases of bubonic plague have been detected in various large harbour cities, such as Glasgow, Sydney, Melbourne, San Francisco, etc., and that over the period 1913-1920 Van Loghem discovered eight cases of rat plague aboard ships in Amsterdam harbour. That in none of these instances an epidemic resulted must indubitably be ascribed primarily to the effective measures instituted by *Homo militans*.

As a second example of an invisible form of life that even in recent times, and with a good chance of success, has threatened human life I mention influenza whose causative agent has turned out to be a virus that only very recently has been isolated by Stanley and made

visible with the aid of the electron microscope. Thus it has revealed itself as a minute spherical particle with a diameter of 0.0001 mm. Many of you may remember the epidemic of 1918 which also in our country carried off a large number of persons in the prime of life. But it is possible that you may not realize that this tragic event was but a small part of a world-wide pandemic that attacked 500 million people, and killed 15 million. During the height of the disease the death toll amounted to one for every fifty individuals per month, a death rate never before encountered. In the U.S.A. 500,000 persons succumbed in the course of 4 months, a number considerably in excess of the total American losses during the second world war.

In connexion with these figures the American scientist, Stanley, remarks:

'I have never been able to understand mankind's resigned and complacent acceptance of such a major catastrophe as the 1918 outbreak of influenza. True, the cause of influenza was unknown in 1918, but one would think that such a calamity would result in a great public demand for a concerted effort to discover the cause of the disease and to develop methods of protection. But this great destruction of human life was accepted almost as ancient peoples accepted natural catastrophes as acts of the gods, and hence beyond the control of man.'

Stanley then goes on to describe how not until more than twenty years later, as a result of the war effort, the American 'Committee on Medical Research of the Office of Scientific Research and Development' was supplied with the means to carry out large-scale investigations. We owe to these studies not only our visual acquaintance with this minuscule scourge of mankind, but also its mass-cultivation in chick embryos, and the transformation of the virus so obtained into a protective vaccine. Even though the situation is greatly complicated by the existence of a number of different types of influenza, Stanley nevertheless does not hesitate to claim that man now possesses the means to prevent forever a recurrence of influenza as one of the major destroyers of human life.

Even to-day dangers continue to threaten. As proof for this statement I mention the cholera epidemic in Egypt where the first cases were reported on September 23 of last year. In the beginning the situation looked grave; the epidemic rapidly spread over all of Egypt.

But *Homo militans* instantly intervened, and particularly by virtue of the aid rendered by the 'World Health Organization' it was possible to vaccinate 80 per cent of the population on short notice. Thereafter the epidemic came to a halt; after December 8 no new cases have been recorded. Through the 'World Health Organization' the Egyptian Government received more than 6.5 thousand litres of vaccine in a short time; 19 different countries, among which the U.S.A., the U.S.S.R., China, and Korea, had contributed their share. Although the epidemic still made 10,000 victims, it must be admitted that this figure compares favourably with the 34,945 deaths caused by the previous Egyptian cholera epidemic of 1902.

Next I want to document my earlier statement that pestilences like those just discussed occasionally have had a decisive influence on the history of mankind. The data have been derived from the exciting book, 'Rats, Lice and History', written by the American bacteriologist, H. Zinsser, and characterized by its author as a biography of typhus.

In an introductory chapter Zinsser pleads for his view that extensive epidemics, no longer diagnosable with certainty, have materially contributed to the down fall of the Roman Empire. We have, for example, learned of descriptions of an epidemic that started in 165 A.D. in Verus' armies in the East, and in a short time spread from Rome throughout the entire domain, 'from Persia to the borders of the Rhine'. Orosius states that several Italian cities and villages were evacuated and fell into decay as a result of the disease. Certain it is that this terror lasted for at least fourteen years, and disappeared only to reappear nine years later, Dio Cassius mentioning that in Rome alone 2,000 deaths occurred daily. It is understandable that under these circumstances, causing the depopulation of cities, and the virtual cessation of agriculture and of commerce, the Roman political apparatus was strongly affected, and cancelled several military campaigns. In the year 250 a new epidemic swept through Rome, with different symptoms and great contact infectivity, ultimately spreading from Egypt to Scotland, and lasting for at least fifteen years. Hieronymus attests to the fact that the human race had virtually ceased to exist, and that the earth had returned to a state of primeval forests and deserts.

I shall skip various minor pestilences, and only mention the great plague epidemic that hit the Roman Empire during the reign of Emperor Justinian in the sixth century. Procopius has left us a dramatic account of the situation in Byzantium, where the death rate rose to 10,000 persons a day, so that it became impossible to bury all the corpses. Frequently these were placed on board ships which were then abandoned to the waves. In numerous Italian provinces the soil remained uncultivated, and under the threefold pressure of pestilence, famine, and the advancing Lombards the Roman Empire perished.

One also finds in Zinsser's book a wealth of data on campaigns and wars, during which the commander of the conquerers won great acclaim, although in reality the battles were determined by disease among the conquered; I shall merely indicate a few of these instances.

Firstly I want to mention the important rôle played by typhus in the siege of Naples in 1528, where the remnants of the Spanish army that had previously and triumphantly plundered Rome, and made Pope Clement VII a prisoner, had taken refuge against the victoriously advancing French army. Philbert, Prince of Orange, who was in command of the Spaniards, wrote to Charles V that the situation was desperate, and that the garrison would not be able to hold out for a month. This would have been of decisive influence on the history of Europe; both Italy and Clement VII would gladly have accepted Francis I as liberator and preserver of the faith. But in the meantime typhus broke out among the besieging French troops, and this to such an extent that within a month only 4,000 men remained of the initial 25,000. The result was that the besieged launched an attack, and destroyed the remnants of the French army. Charles V had conquered decisively; Italy fell completely under Spanish domination; and Clement VII surrendered. In 1530 he crowned Charles as Holy Roman Emperor in Bologna; the virus of typhus had willed it so.

Zinsser furthermore remarks that the thirty-year war can best be characterized as the most gigantic epidemiological experiment ever perpetrated on mankind. Europe exhibited the picture of a continuous series of outbreaks of every imaginable infectious disease. For thirty years armies wandered about in the realm; smaller gangs, fugitives, deserters roamed around at large. Famine was the result, and entire populations migrated in hords, in search of food and shelter. And wherever man went, everywhere was he followed by disease. It

is clear that under the circumstances the outcome of the conflict was not determined by the strength, courage, or leadership of the contesting armies, but by the invisible organisms, or, if you wish, by chance. This is strikingly illustrated by the following episode. In 1632 Gustavus Adolphus and Wallenstein faced each other in the battle for Nuremberg. Typhus, however, intervened, killed 18,000 soldiers, and the result was that both armies, without engaging in battle, rapidly retreated in order to escape the pestilence.

Although generally the origin of the Haitian Republic is ascribed to the genius of Toussaint l'Ouverture, in fact it was the yellow fever virus that was responsible. In 1801 Napoleon dispatched an army of 25,000 men to suppress the revolt of the negroes. Toussaint was defeated on short notice, and escaped into the interior; soon afterwards yellow fever broke out among the French soldiers; no less than 22,000 men fell victim to it. The sorry remnants could do nothing but evacuate the island in 1803.

The book about Napoleon's Russian campaign, written by the Chevalier de Kerckhove who accompanied the expedition as a high-ranking medical officer, hardly leaves any doubt that the destruction of the 'Grand Army' should be ascribed to typhus and dysentery rather than to the resistance of the Russians.

In a short time the army of 500,000 men assembled by Napoleon in 1813 suffered a loss of 219,000 from disease alone. It seems unequivocal that Napoleon's power in Europe was primarily broken by disease, and not by military prowess.

It is not possible to conclude that in the Crimean war disease caused the balance of power to swing in favour of one side, but because in this case dependable statistics have been assembled for the first time, it is certain that the losses of all combatants due to typhus, cholera, and other diseases exceeded by many times those suffered in battle.

Perhaps it will be felt that these matters pertain largely to the distant past, and that nowadays things are not as bad. One might use as examples the two world wars that are still fresh in our memory, and that definitely have not left the impression of large-scale diseases.

But then it must be realized that if in the present day and age *Homo militans* marches out to settle mutual differences by force of arms, he fully reckons with the fact that a large part of his activity must be devoted to a war against the ubiquitous and invisible third. Only in

this way can it be understood that in the first world war the opposing armies have succeeded in keeping the Western Front free from typhus, owing to an energetic delousing policy. If we follow Zinsser, however, typhus had a not insignificant share in the victory of the Allies because the epidemic which broke out in Serbia almost immediately after the start of the war, and in six months made 150,000 victims, deterred the Austrian army from invading the country. Such an invasion, and its consequences for the fate of the other Balkan countries, would in all probability have greatly benefited the Central powers.

With respect to the second world war it is still too early to draw up a balance concerning the influence of invisible life on the course of events. But many of our compatriots who were carried off to Germany have experienced that especially during the final chaotic year typhus reared its ugly head, albeit that *Homo militans* succeeded in localizing the effects of this calamity.

It would please me if from these rather macabre examples – which, by the way, could have been amplified by numerous others – my audience had become convinced that in effect the course of history has not infrequently been governed by the invisible living world, and that a perpetual alertness of *Homo militans* is imperative if in future man desires to keep his terrestrial existence free from such influences.

One might, however, counter that the previous discussion refers only to periods of war and chaos, whereas during more normal times one can live safely behind the hygienic barriers erected by our sanitary services, at least as far as Western Europe is concerned. But then I must reply that some invisible organisms do manage to break through these barriers. This applies, for instance, to *Mycobacterium tuberculosis*, the causative agent of tuberculosis, often designated as ‘Public Enemy Number One’. Also in our country there are many who succumb to this cruel disease or who are incapacitated by it for long periods of time. Even though the final goal has not been achieved by any means, it is fortunate that we may conclude that *Homo militans* has long been active in this field, and I may here be permitted to refer to his latest combat weapon.

Remembering the adage that thieves are the best agents for apprehending thieves, and impressed by the successes scored with penicillin, attempts have been made to use the fighting equipment with which

various microbes mutually attack each other in this case as well. Following the isolation of *Actinomyces griseus*, antagonist of *Mycob. tuberculosis*, it became possible to isolate also the chemical entity, now designated as streptomycin, which the actinomycete uses to maintain itself in the struggle for existence. Even though streptomycin has been found not always to be entirely innoxious to human beings, surprisingly good results have already been achieved with it in cases of some special types of tuberculosis.

There is yet another matter I wish to call to your attention. It might be felt that by now the microbiologist should be in possession of a complete catalogue of all invisible forms of life that may directly threaten man's health, thus eliminating the element of surprise in the course of the subsequent fight against disease. The reply is, however, that this notion is incorrect, and that during recent years diseases have appeared of which it may be assumed on good grounds that they did not formerly exist.

Fortunately we can immediately counter that there are equally sound reasons for believing that other infectious agents, which in previous centuries have caused great ravages, have meanwhile died out. As an example of the latter I may mention the 'English sweating sickness', first described in 1485, but about which nothing more has been heard since 1552.

The first epidemic broke out after the battle of Bosworth; it led to a disbanding of the victorious troops under Henry VII, whose coronation consequently had to be postponed. The disease spread over all of England; the mortality was very high; only one of every 100 persons attacked escaped with his life. Later epidemics occurred in 1507, 1518, 1529, and 1551; partly these spread even as far as the continent. Thereafter no one has ever again heard of a disease with symptoms such as those attending the sweating sickness. A similar situation is encountered in the case of a somewhat related, but nonetheless clearly distinguishable disease, known as 'la suette des Picards', because it was in Picardy that it first occurred.

After this excursion into the realm of vanished diseases I return to the subject of the 'new' ones, which implies a warning that the future fight against invisible life should not be underestimated.

In this connexion some introductory remarks are in order anent the origin of parasitism. It appears that there are but few disease-

provoking agents that do not show an unmistakable relationship with forms that occur in a free-living state in nature, the so-called saprophytic types. In view of this fact we are led to ascribe the appearance of parasites to the occurrence at some time of an event whereby some of these saprophytes, exposed as are all living organisms to gradual modifications, have succeeded in overcoming the natural resistance that every living cell complex offers against invasion by species-foreign life. There is every reason to assume that this process has started in the dim past of the developmental history of life on earth. In the case of very ancient fossils, dating back to the Permian (200 million years ago) the palaeontologists have found clear-cut indications of infectious diseases, and some scientists are even inclined to ascribe the extinction of numerous animal species during prehistoric times to such diseases.

Now it seems far from improbable that a similar transformation of a saprophytic into a parasitic mode of life may occasionally occur even to-day. This possibility is increased by the fact that in recent years the microbiologist has managed to induce mutations in numerous microbes with the aid of irradiation, and it is readily conceivable that similar effects are produced under natural conditions by cosmic rays.

Furthermore, we have to count with the possibility that during the repeated transmission from host to host the uniformity of the milieu may cause modifications of the parasite that may have either good or bad consequences.

Whereas the first invasion generally leads to a violent reaction, causing either host or invader to succumb acutely, a modicum of adaptation is prone to induce in the host a chronically proceeding alteration; and eventually a symbiosis, or coexistence with mutual benefit, may be established in which the host no longer shows any signs of damage. I may here refer, for example, to the well known spirochaetosis of mice; and our fellow member, Swellengrebel, has discovered a sensational example in human pathology when he established that particularly the younger generation of aborigines in Surinam was often strongly infected with virulent malaria parasites without apparently suffering any ill effects.

Such an adaptation may, however, equally well lead to other consequences. In numerous cases it has been found that passage through

a species-foreign host drastically modifies the properties of viruses. Thus, in monkeys the virus of yellow fever causes symptoms that show a close resemblance to those of the human disease. Inoculated into mice, the same virus causes a form of encephalitis that can be transferred in series in this animal. But if now the virus is reinoculated into monkeys, it is not yellow fever that results; the virus retains its neurotropic characteristics.

This clearly shows that at any time we may be confronted with new disease symptoms of man without this having to be ascribed to a *de novo* creation of new parasitic forms.

Besides, there exists another source of new diseases for man, *viz.*, an initiatory contact with forms of parasitism that have become established elsewhere in the animal kingdom. Such a contact always offers the opportunity of a transmission to a human host. A typical instance of a new virus disease that appeared in this manner is psittacosis, first described by Ritter in 1879, in which cockatoos or parakeets undoubtedly serve as the reservoir of the virus which is extremely contagious and highly pathogenic for man.

A second instance of a disease that probably originated recently was first described in 1910 as the 'ground squirrel disease' in Tulare County, California. The causative agent turned out to be a hitherto unknown bacterium, similar to though clearly distinguishable from the bacterium of bubonic plague; it has been named *Pasteurella tularensis*. Nine years later, and in a distant section of America, Utah, a party that had been hunting wild rabbits and had skinned part of the booty for food, took ill. In both the patients and the rabbits *P. tularensis* was found. Since that time the disease, now known as tularemia, has been encountered not merely in many parts of America, but also in Norway, Sweden, the U.S.S.R., and Japan, often in the form of epidemics.

The causative agents of these two new diseases, against which a wholesale defense is still in its infancy, have been specifically included in the experimental investigations, initiated during the war in Camp Detrick, U.S.A., on the prevention of the spread of contagious diseases.

Although we may fervently hope that man will be spared the disgrace of bacteriological warfare, we Hollanders should feel humiliated if we remember that it was one of our compatriots who was probably the first to advocate this kind of warfare. It was Pieter van Woensel,

born in Haarlem two centuries ago as the son of a physician, who in 1790 published his 'Travel Notes', and described his experiences gathered during a sojourn of five years in Turkey, to which country he apparently lost his love. Anxious about a Russian threat against Constantinople, he developed in his book a method of defense based upon the possibility of artificially spreading bubonic plague among the inhabitants of an aggressor country. It is hard to decide which is the more striking in Van Woensel's narrative; the diffidence with which he develops his idea, or the almost lyrical commentary devoted to the efficacy of the defense measure advocated.

While I have thus far only spoken of the influence exerted by disease-producing representatives of the invisible world on humanity and its activities, other and in themselves quite innocent microbes have not remained entirely without effect. A striking example may be found in the so-called 'miracle bacterium', nowadays known under the scientific name of *Serratia marcescens*. This ubiquitous species possesses the property of producing a red pigment in consequence of which a localized development of the bacterium leads to the formation of red-coloured patches, showing an unmistakable resemblance to a blood spot. Particularly favourable substrates for multiplication of this organism are starchy foodstuffs such as bread and polenta (corn-meal mash).

It is not surprising that at a time when the natural origin of such 'blood' formation was unknown, superstition attached far-reaching significance to this phenomenon. In old chronicles many entries can be found concerning the consequences; a summary of these reports can be found in publications by Ehrenberg, Scheurlen, and Harrison. The reactions of the human mind to the occurrence of 'blood', especially if this appeared on the eucharist, were quite divergent. Sometimes it was accepted as a miracle, and thus led to an enhanced devotion of the faithful. Raphael's famous painting, 'The Miracle of Bolsena', attests to this type of effect. Not less frequently, however, was the phenomenon interpreted differently, and was blamed on the unbelievers, particularly among the Jewish population. Repeatedly these have had to pay for it by hundreds being burned at the stake, and this has tempted Scheurlen to the remark that the wholly innocent miracle bacterium is responsible for more deaths than many a pathogen. In 1819 an epidemic occurrence of red discolouration of diverse

food products in various communities in the neighbourhood of Padua resulted in a systematic investigation by several Italian scientists, among whom Bizio was the first convincingly to show that the pigment production was the result of the growth of a minute plant-like organism. Since that time the appearance of the 'miracle bacterium' has been virtually eliminated from the realm of superstition.

The history of this organism shows that a microbe may also exert a mental influence on man that should not be underrated. That this is equally true for pathogenic germs, and that consequently they can leave a lasting imprint on the individual psyche, besides causing the mass effects mentioned earlier, will be understood by any one who has taken notice of Albert Camus' exciting book, 'The Plague', which has rightly attracted a great deal of attention among the post-war French literary accomplishment. It must, of course, be kept in mind that here we enter the realm of fiction, and that Camus' description of the plague epidemic and its effects can be severely criticized as far as the medical and hygienic implications are concerned. This, however, does not detract from the literary merits of the book, particularly because the author leaves open the possibility that we should interpret his description of life in a city beleaguered by plague, and closed off from communication with the rest of the world, as a transposition of life in a region groaning under the yoke and terror of foreign occupation. Nevertheless, the reactions of Camus' characters to a perpetual and inescapable threat to life seem so completely acceptable that there is every reason to conclude that similar mental reactions will be experienced during real epidemics.

Approaching the end of my discourse it can no longer be denied that an element of deception has crept into the choice of the title of my lecture. It is true that I have had an opportunity to stress the notion that in human history *Homo militans* plays an increasingly large rôle in the incessant combat with extra-human forms of life. But I have failed to speak of this type of man, and of the incentives that guide him in his struggle. A profound analysis of the life histories of those who have fought or are fighting in the front ranks might open up important vistas. This I shall not attempt; I shall restrict myself to a few disconnected remarks.

Certain it is that we may encounter *Homo militans* in a great diver-

sity of forms. Firstly there is the man who, vexed with the damage that humanity has to suffer from some forms of life, finds his vocation in fighting it. A typical example is the American specialist, J. L. Nicholles, who in his recent book, 'Vandals of the Night', has reported on his life-long war against the rat, and can boast of having eliminated no less than 25 million individuals of this foe of humanity. We may here remark, too, that this veteran is anything but optimistic concerning the outcome of the fight. On the basis of his experience he has become so impressed with the enormous adaptive capacity of his enemy that he concludes that there is at least a fair chance that the rat may out-live man.

Related to these specialist-fanatics, yet different in some respects, are the fighters who do not so much direct their efforts towards particular enemies, but more generally have taken their task to be a protection of the food provision of mankind against any conceivable assailant. They are marshalled by generals who have at their disposal extensive general staffs and adequate organizations for the execution of the measures they deem necessary.

The commanders are often phytopathologists or economic entomologists; the earlier mentioned entomologist, Howard, is a typical case in point. Among the co-militants we encounter, however, also sober statisticians, geographers, sociographers, meteorologists, and many other scientists who may never have been confronted with the assailants of our foodstuffs and the damage they cause. Finally there is the army of executors among whom can be found both sharpwitted biologists and chemists, and agriculturalists, controllers, and airplane pilots.

Apart from these we meet *Homo militans* in the form of those who are engaged in mortal combat with the invisible forms of life that directly threaten the health and continued existence of humanity in a serious manner. Here too the struggle has long ago resulted in the formation of powerful organizations, spread out over the entire world, and which comprise many fighters who are not directly associated with the medical profession *per se*. Of the leaders in this combat, the 'men against death' as they have aptly been characterized by De Kruif in one of his books, it may again be asserted that they have been caused to take up the fight by many divergent motivations. On the one hand there is the pure-blooded scientist, the scientifically

possessed, who wants to comprehend everything, and includes in his search for understanding the problem of why man sometimes falls prey to invisible powers. One may in this connexion think of such a man as Pasteur, and I believe that I shall not detract from the invaluable merits of this great benefactor of mankind if I claim that for this type of fighter the notion of therapy and prevention always comes in the second place.

Then there are those who are pushed into the fight by rebelling against or compassion with the suffering of his fellow-men; by the belief that they should not acquiesce in his downfall. Semmelweis, the conqueror of childbed fever, is a typical representative of this variant of *Homo militans*. And among the less prominent fighters there are many who, though less impassioned, nevertheless have dedicated themselves, driven by a conscious effort to serve suffering humanity. Certainly this group includes those exceptional persons who have occasionally made themselves available as human experimental animals in the study of mortal diseases. The American military personnel who, in 1900, thus permitted Reed in Cuba to solve the problem of yellow fever provide the best known, though by no means the only example.

And lastly, besides these individuals who consciously sacrificed themselves 'for the cause of humanity and in the interest of science', we may not forget the many to whom probably applies what Camus in his earlier mentioned book puts into the mouth of one of his improvised plague fighters. When sounded out about the motives of his terribly risky cooperation, the answer is hesitantly summarized in the simple words, 'par honnêteté'.

I realize that these few remarks are anything but sufficient to form a suitable image of *Homo militans* such as he appears to-day. Nevertheless, it would already give me great satisfaction if I had succeeded in imbuing my audience with the notion that it is the highest time that the *homines militantes* of this earth cease to exhaust themselves in infamous mutual combat, and instead dedicate themselves to the more urgent and at the same time more glorious task of ensuring the existence of humanity by protecting it against its many beleaguers.

In that event you may be found willing to reserve in the human family circle a lasting place not only for *Homo sapiens*, *Homo faber*, and surely, *Homo ludens*, but also for *Homo militans*!

HOMO MILITANS

TOEN de Hollandsche Maatschappij der Wetenschappen in 1901 besloot voortaan op het programma van haar Algemene Vergaderingen een plaats in te ruimen voor een mededeling over een wetenschappelijk onderwerp, werd tevens bepaald, dat dit onderwerp bij beurten uit verschillende vakken zou worden gekozen. Dit nu brengt voor het lid, dat het voorrecht heeft een der jaarlijkse spreekbeurten te mogen vervullen, onmiskenbaar ook een aanmerkelijke verantwoordelijkheid mede ten opzichte van het vak, dat hij vertegenwoordigt. Immers de wijze, waarop hij zich van zijn taak kwijt, zal allicht niet geheel zonder invloed blijven op de plaats, welke zijn wetenschap zal innemen in de waardering van Directeuren en mede-Leden.

Op mij als microbioloog rust dan de taak de belangstelling van mijn toch grotendeels uit leken bestaand auditorium wakker te roepen voor onze kennis betreffende de wereld der microben.

Ik heb dus gemeend er naar te moeten streven mijn auditorium 'microbe-minded' te maken, althans voor de periode tot de volgende jaarvergadering, waarop weer nieuwe indrukken de huidige zullen verdringen. Dit bracht mij ertoe een poging te wagen U het besef bij te brengen, dat hoezeer de meesten Uwer zich tot dusver wel hebben bevonden bij een negatie van de microbenwereld, de microben nu eenmaal niet altijd bereid zijn de mensheid te negeren. Met andere woorden het leek voor mij een aantrekkelijke taak een beknopte beschouwing te wijden aan de verhouding mens-microbe.

Bij nadere overweging werd het mij evenwel spoedig duidelijk, dat binnen het gegeven bestek een alzijdige belichting van deze verhouding niet wel mogelijk zou zijn en ik mij dus tot enkele aspecten zou moeten bepalen. Ik had U nu b.v. kunnen spreken over de microben als weldoeners der mensheid en hierbij kunnen trachten U de troosteloze verschrikkingen af te schilderen van het menselijk leven op een microbenloze aarde. Maar liever dan dit te doen, heb ik gemeend Uw aandacht te moeten vragen voor de zoveel minder talrijke aan de mens vijandige microben. Enerzijds zijn het toch juist deze microben, welke

rechtstreeks een stempel op de ontwikkelingsgeschiedenis der mensheid hebben gedrukt, anderzijds scheen een bespreking daarvan de gelegenheid te openen mede Uw belangstelling te wekken voor de mens in zijn nimmer aflatende strijd tegen zijn belagers uit de wereld van het onzichtbare leven.

Het was in deze phase van mijn overpeinzingen dat ik besloot tot de titel *homo militans* voor mijn voordracht. Spoedig hierna trok het mijn aandacht, dat enkele jaren geleden een boek onder dezelfde titel was verschenen. Kennismaking met dit geschrift deed mij besluiten mijn onderwerp in een wat ruimer kader te plaatsen. In bedoeld boek, dat even voordat ook ons land in de oorlog werd betrokken het licht zag, tracht de bekende psychiater Meerloo een analyse te geven van de menselijke factoren, welke tot het ontstaan van oorlogen leiden.

In het bijzonder trof mij intussen bij de lectuur van Meerloo's boek de volgende zinsnede:

'Wordt niet alle onderlinge strijd opgeheven door een nog grooter gevaar van buitenaf? Bij menschen en bij landen? Als Mars ten oorlog trok tegen moeder Aarde, zouden de Vereenigde Staten der Wereld een voldongen feit zijn.'

Nu wil het mij voorkomen, dat niettegenstaande de recente ontwikkeling der radar-techniek een eerste contact met de maan heeft tot stand gebracht en de eerste aardse raketten haar wellicht spoedig zullen bereiken, er vooralsnog weinig uitzicht op een interplanetaire oorlog bestaat. Indien dus de samenwerking der mensheid hierop zou moeten wachten, moeten de perspectieven hiervoor wel weinig gunstig worden beoordeeld.

In dit verband dringt zich evenwel de vraag op, of de gevaren, welke de mensheid in haar geheel bedreigen, juist altijd van buitenlandse herkomst behoeven te zijn, waarbij zich dan dadelijk de tweede vraag aansluit, of de mens los hiervan inderdaad de onbedreigde heerser van de aardbol is, die hij zich doorgaans waant te zijn.

En als deze laatste vraag ontkennend zou moeten worden beantwoord, is het dan niet aangewezen, ja noodzakelijk, dat de mensheid zich op korte termijn aaneensluit om gezamenlijk de dreigende gevaren af te wenden?

Gezien in het licht van het voorafgaande lijkt het lonend het besef wakker te roepen, dat voor de mensheid in haar geheel inderdaad ernstige gevaren bestaan. Ten volle moet er toch rekening mede wor-

den gehouden, dat op aarde naast de mens nog uiterst talrijke andere levensvormen om hun voortbestaan worstelen. En waar leven leven ontmoet ontstaat strijd, strijd om ruimte, doch bovenal om voedsel. Te weinig wordt beseft, dat ook de mens volop in deze strijd is betrokken en dat de goede uitslag veel minder zeker is dan de grote meerderheid der onwetenden blijkbaar stilzwijgend aanvaardt.

Ik wil dan trachten enkele episoden uit deze strijd, die de mensheid van haar geboorte af heeft moeten strijden, voor U te belichten. Hierbij zal uiteraard het accent vallen op de strijd tegen het onzichtbare leven, de microbenwereld dus, maar anderzijds mag het beseft, dat ook hoger georganiseerde wezens een reële bedreiging vormen, hierbij niet ontbreken.

Slechts één vijand van *homo sapiens* – wellicht de verschrikkelijkste van allen – namelijk zijn broeder *homo ignorans* wil ik hier welbewust buiten beschouwing laten. Wie diep onder de indruk wil komen van de wijze, waarop de mens zelve door onwetendheid en onnadenkendheid bezig is zijn bestaan op aarde te ondermijnen, die leze het onlangs verschenen boek van Fairfield Osborne, getiteld: 'Our Plundered Planet'. In dit geschrift, dat is opgedragen 'to all who care about tomorrow', zet de vermaarde voorzitter van de 'New York Zoological Society' op overtuigende wijze uiteen hoe de mens tengevolge van de zo beangstigend snelle toeneming van het bevolkingsaantal in steeds sterkere mate roofbouw is gaan plegen en daardoor geleidelijk is geworden tot een 'geologische kracht', welke reeds thans hard op weg is het eens zo bloeiende aanschijn van onze planeet tot dat van het doodse maanlandschap te laten naderen.

In dit verband is het leerrijk ons de cijfers voor de groei der aardse bevolking even scherp voor ogen te stellen: naar schatting bedroeg het totale bevolkingscijfer in 1630 400.000.000, welk aantal omstreeks 1830 zou zijn verdubbeld en in 1900 andermaal verdubbeld. In 1940 was de 2 milliard zeker overschreden, terwijl bij handhaving van de thans bestaande bevolkingsaanwas van 1% per jaar omstreeks 2010 de 4 milliard zal worden bereikt!

Men zou nu geneigd kunnen zijn uit deze gegevens te besluiten, dat de mens het er in zijn strijd met het buitenmenselijk leven toch maar redelijk wel heeft afgebracht, maar men zal goed doen te beseffen, dat juist de problemen, welke gekoppeld zijn aan deze enorme stijging

van het mensenaantal de activiteit van *homo militans* in toenemende mate opeisen.

Wanneer ik dan nu er toe overga enkele vijanden der mensheid de revue te laten passeren dan zal men hierbij behalve aan de onzichtbare ééncellige organismen wel vooral aan dierlijke organismen denken. Zij, die het leven temidden der weelderige tropische vegetatie kennen, zullen evenwel beseffen dat althans de voortbrengselen der menselijke cultuur ook aan voortdurende aanvallen van hogere plant-aardige organismen bloot staan.

Ik twijfel er niet aan of voor menigeen, die als ik het voorrecht had om vóór 1940 de bootreis van Nederland naar Insulinde te maken, zal de grote ongereptheid der machtige tropische natuur de eerste indruk van dit schone land zijn geweest. Mij althans trof gedurende de destijds nog een tweetal dagen vragende reis van Padang naar Tandjong Priok niets zo zeer als de dicht aaneengesloten, weelderige groene vegetatie, waarmede de Westkust van Sumatra en de eveneens gepasseerde kleinere eilanden waren bedekt. Geen spoor van menselijke bewoning viel waar te nemen en zo men al op verstandelijke gronden moest besluiten, dat hier en daar de mens wel eens moest zijn doorgedrongen, was hij er toch kennelijk niet in geslaagd blijvend zijn stempel op het landschap te drukken. Afgaande op de uitspraken van onze Indië-vliegers, die bij het overtrekken van Sumatra over de monotonie van hun uitzicht klagen en in dit verband zich van de kernachtige karakterisering 'boerenkool' bedienen, heeft deze situatie ook heden ten dage nog geen verandering ondergaan. Eenmaal op het zo dichtbevolkte Java aangekomen wijzigde de aanvankelijke indruk zich weliswaar snel. Hier toch bepaalden de steden, de weliswaar verspreide, maar toch welhaast alomtegenwoordige landelijke behuizing, de uitgestrekte sawah's der bevolking alsmede de onder Westerse leiding staande cultuurondernemingen voor een belangrijk deel de physionomie van het landschap.

Maar toch drong het al spoedig tot mij door, dat deze menselijke penetratie in de tropische levende natuur niet slechts dank zij veel energie tot stand was gekomen, maar ook alleen ten koste van veel strijd kon worden gehandhaafd. Treffend stond mij dit voor ogen, toen ik op een excursie in het Bantamse stond voor enige door plantengroei overwoekerde ruïnes, waarop alleen de met moeite teruggevon-

den inscriptie 'V.O.C.' nog aanduidde, dat hier onze voorvaderen hun versterkingen bouwden en bewoonden. Het was onmiskenbaar, dat er niet zo heel veel tijd meer zou verlopen alvorens de laatste sporen van menselijke beleving hier zouden zijn uitgewist.

Maar dit simpel getuigenis zal op mijn auditorium zeker niet veel indruk vermogen te maken. Laat ik daarom liever een ogenblik Uw aandacht vragen voor de beschrijving van iemand, die geheel overeenkomstige indrukken op meesterlijke wijze onder woorden heeft gebracht.

Het is Pierre Loti, die in 'Un pèlerin d'Angkor' rapporteert over zijn in 1901 ondernomen reis naar Angkor, in het binnenland van Indo-China, waar – sedert door de Fransen gerestaureerde – ruïnes van een ontzaglijke omvang getuigen van het diep in de eeuwen teruggaande trotse rijk der Khmers. Hier vestigde zich een Brahmaanse beschaving, die een overweldigend groot aantal rijk met beeldhouwwerk versierde tempels schiep, welke beschaving evenwel op haar beurt door een Bouddhistische cultuur werd verdrongen en veredeld. Loti schrijft hiervan: 'Il semble que, sous le bouddhisme, la ville d'Angkor connut l'apogée de sa gloire. Mais l'histoire de son rapide et mystérieux déclin n'a pas été écrite, et la forêt envahissante en garde le secret. Le petit Cambodge actuel, conservateur de rites compliqués au sens perdu, est un dernier débris de ce vaste empire des Khmers, qui depuis plus de cinq cents ans a fini de s'éteindre sous le silence des arbres et des mousses . . .'

Loti geeft een levendige beschrijving van de lange reis, welke hij moest afleggen alvorens hij eindelijk de dicht begroeide muren van het oude Angkor-Thom aanschouwde. Door een sombere, met monsterachtige Brahma-figuren overdekte poort trad hij binnen, maar ook hier zette het door slangen en apen bevolkte oerwoud zich voort, temidden waarvan evenwel de ontelbare hier en daar met de plantengroei als het ware versmolten overblijfselen van architectuur en sculptuur aan de oude grootheid herinnerden.

Hier moge ik Loti nog een ogenblik zelf aan het woord laten:

'Car il y a un entêtement de destruction même chez les plantes. Le Prince de la Mort, que les Brahmes appellent Shiva, celui qui a suscité à chaque bête l'ennemi spécial qui la mange, à chaque créature ses microbes rongeurs, semble avoir prévu, depuis la nuit des origines, que les hommes tenteraient de se prolonger un peu en construisant

des choses durables; alors, pour anéantir leur oeuvre, il a imaginé, entre mille autres agents destructeurs, les pariétaires, et surtout ce "figuier des ruïnes" auquel rien ne résiste.'

'C'est le "figuier des ruïnes" qui règne aujourd'hui en maître sur Angkor. Au-dessus des palais, au-dessus des temples qu'il a patiemment désagrégés, partout il déploie en triomphe son pâle branchage lisse, aux mouchetures de serpent, et son large dôme de feuilles.'

Zeker hebben naast de tropische plantengroei andere factoren medegewerkt aan deze geheimzinnige afbraak van een der meest grootse uitingen van de menselijke cultuur. Maar dit neemt niet weg, dat hier toch wel heel treffend gedemonstreerd is hoe vergankelijk het mensenwerk is, dat zelfs in zijn meest duurzame vormen slechts door voortdurende strijd tegen het niet-menselijke leven kan worden gehandhaafd.

Terloops moge worden opgemerkt, dat het voorafgaande er zich zeker mede toe leent een nieuw licht te werpen op het 'Je maintiendrai', het fiere devies der Oranjes, wellicht in zijn verzet tegen het *παντα ῥέει* de meest trotse leuze van alle tijden.

Het is welhaast overbodig er op te wijzen, dat uitingen van de menselijke cultuur nog wel aan andere bedreigingen door niet-menselijke levensvormen bloot staan dan waarvan sprake was in het zo juist besproken extreme geval van verwoesting van machtige bouwwerken door de tropische vegetatie. De meeste dezer uitingen toch zijn van veel brozer natuur. Men denke slechts aan schilderstukken, waarvan de organische basis een alleszins aanvaardbaar voedings-substraat vormt voor tal van insecten, schimmels en bacteriën. Het is verheugend te mogen vaststellen, dat, toen het oorlogsgevaar zich hier te lande deed gevoelen, de verantwoordelijke autoriteiten aan dit gezichtspunt tijdig aandacht hebben geschonken door de schilderijen en tapisserieën onzer musea onder te brengen in ondergrondse ruimten, waarin door regeling van temperatuur en vochtigheid de genoemde belagers in bedwang konden worden gehouden.

Een veel meer rechtstreekse bedreiging van de mensheid is evenwel gelegen in de omstandigheid, dat haar voortbestaan gebonden is aan een onafgebroken toevoer van voedsel, waarvan de landbouw uiteindelijk de bron is. Het spreekt vanzelf, dat onze cultuurgewassen eveneens aan tal van andere levensvormen een bestaansbasis bieden

en dat dientengevolge zowel de gewassen op het veld, als de daarvan geoogste producten bij opslag voortdurend aan aanvallen van het niet-menselijke leven bloot staan. Er kan nu niet genoeg de nadruk op worden gelegd, dat in dit opzicht de gevaren in de laatste eeuw enorm zijn gestegen. Het zo sterk toegenomen bevolkingscijfer heeft er toe geleid, dat een voortdurend groter deel der aardoppervlakte in cultuur is gebracht, tengevolge waarvan de vegetatie een zoveel eenzijdiger karakter heeft verkregen. Dit nu brengt mede, dat plagen, welke eenmaal vaste voet verkrijgen in bepaalde cultuurgewassen, zich min of meer lawine-achtig zouden kunnen uitbreiden, waaruit een catastrofie voor de mensheid zou kunnen resulteren.

Reeds in 1915 sprak de Amerikaanse entomoloog Johnston uit, dat de volgende oorlog er een zou zijn tussen mens en insecten en hoewel deze profetie slechts ten dele in vervulling is gegaan, kan niemand, die kennis neemt van het in 1931 verschenen boek van L. O. Howard, getiteld: 'The Insect Menace', zich onttrekken aan het besef, dat het uitermate noodzakelijk is, dat de generale staf der mensheid zich in sterkere mate dan tot dusver concentreert op de oorlog tegen de insecten.

Howard, die gedurende lange jaren aan het hoofd stond van het 'Bureau of Entomology of the U.S. Department of Agriculture' wijst er in de eerste plaats op, dat de palaeontologische gegevens geen twijfel laten, dat insecten zich zeker reeds 40.000.000 jaren – waarschijnlijk zelfs nog veel langer – op de aarde handhaven en dat terwijl in deze periode zovele andere diersoorten radicaal zijn verdwenen. Hiertegenover staat, dat de menselijke bewoning der aarde zich wellicht nog pas over 500.000 jaren uitstrekt en dientengevolge in geologisch opzicht gezien nog slechts een experimenteel karakter draagt. Hierbij komt dan, dat bevoegde beoordeelaren het totaal aantal soorten van insecten, dat heden ten dage de aarde bevolkt, op 2 à 10 miljoen schatten, waarvan zeker nog geen tiende deel door de entomologen is beschreven. Ook al is het aantal soorten, dat zich tot dusver in grote stijl op de mens en zijn voortbrengselen heeft geworpen nog betrekkelijk gering te noemen, dit neemt niet weg, dat men tenvolle rekening moet houden met de waarschijnlijkheid dat onder de nog onbekende soorten vele potentiële vijanden van de mensheid zullen schuilen, die onder invloed van wijzigingen in de cultuurvoorwaarden op ieder ogenblik hun aanwezigheid en hun macht zullen kunnen manifesteren.

Het valt buiten het bestek van deze voordracht – en eveneens buiten mijn competentie – hier een evenwichtig overzicht te geven van hetgeen de economische entomoloog heden ten dage ter bescherming van het mensdom verricht. Een enkel voorbeeld moge evenwel toelichten, welke omvang en betekenis het werk van deze variëteit van *homo militans* reeds heeft aangenomen.

Ik kies daarvoor de sprinkhaanbestrijding, omdat hierbij de noodzaak van internationale coöperatie zo duidelijk aan het licht is getreden. Men behoeft zich slechts de bijbelse beschrijving van de ‘achtste plaag’ in Exodus 10 voor ogen te stellen om te beseffen, dat van oudsher sprinkhaanplagen door tal van volken zijn gevreesd als de voorbode van hongersnood en ellende. Gedurende eeuwen heeft de enige verdediging van de mens bestaan in een hopeloos ondoeltreffende poging tot verdelging van het insect op de plaatsen, waar dit in zwermen neerstreck. Een nieuw moment in de strijd tegen de plaag trad in 1921 in, toen Uvarov zijn phase-theorie over de sprinkhaanontwikkeling opstelde. Deze onderzoeker stelde vast, dat de larven van vele sprinkhaansoorten zich veelal eerst tot de z.g. *solitaria*-phase ontwikkelen, welke zowel in gedaante als in leefwijze sterk verschilt van het insect zoals zich dit in de zwermmende individuen voordoet, en wel in die mate, dat men meende hier met een afwijkende soort te doen te hebben. In deze phase is de aangerichte schade gering en ook de verplaatsing beperkt. Wanneer evenwel de omstandigheden er toe leiden, dat een groot aantal van deze *solitaria*'s in een beperkt voedsel-region wordt samengedrongen, ontwikkelt zich hieruit de ook in morfologisch opzicht belangrijk afwijkende *gregaria*-phase, welke zeer vraatzuchtig is en daarbij sterke neiging tot massale trek vertoont.

Het internationale onderzoek heeft het grote belang van deze ontwikkelingsgeschiedenis voor het vraagstuk van de bestrijding duidelijk doen uitkomen. Zo is b.v. komen vast te staan, dat de zwermen van de Afrikaanse zwermsprinkhaan (*Locusta migratoria migratorioides*), welke in de periode van 1928–1937 niet minder dan 10.000.000 vierkante mijlen in zulke uiteenlopende landen als Brits- en Frans-West-Afrika, de Belgische Congo, Soedan, Eritrea, Brits-Oost-Afrika en Rhodesië teisterden, alle afkomstig waren van de *solitaria*-phase, welke zich bevond binnen een areaal van slechts 10.000 vierkante mijlen in het moerasgebied van de Niger in de Franse Soedan.

Duidelijk is uit verschillende onderzoeken gebleken, dat een con-

trôle op de ontwikkeling van de op zich zelf onschuldige *solitaria*-phase het aangewezen middel is om rampen in ver afgelegen streken te voorkomen. Hiertoe is internationale coöperatie uiteraard onmisbaar. Op de in 1938 te Brussel gehouden 5de Internationale Sprinkhanen Conferentie is dan ook aanbevolen tot dit doel een internationale dienst in het leven te roepen, welk project tengevolge van het uitbreken van de oorlog nog niet is verwezenlijkt. Wel is reeds sinds 1930 in Londen werkzaam het 'Anti-Locust Research Centre', waar o.m. de uit talrijke landen binnenkomende gegevens worden gecoördineerd en dat op grond hiervan voorspellingen en waarschuwingen aan bedreigde streken doet uitgaan.

Gedurende de oorlogsjaren is er op initiatief van de Engelse Regering op wat beperkter schaal reeds belangrijk internationaal werk gedaan inzake de bestrijding van de z.g. woestijnsprinkhaan, waarvan de zwermen de voedselbronnen van Oost-Afrika, het Midden-Oosten en Brits-Indië bedreigden. Genoemde sprinkhaansoort heeft haar kweekplaats in de Engelse Soedan en Eritrea, trekt van hier over de Rode Zee naar Arabië en Noord-West-Indië, vanwaar uit de gehele omgeving van de Perzische Golf wordt geëvenaheerd. Vanuit Arabië worden voorts veelal het dal van de Euphrates, Syrië, Palestina en de Nijl-delta bestookt. Groepen van Engelse, Egyptische en Brits-Indische onderzoekers hebben nu in nauwe samenwerking Arabië onder contrôle gehouden, terwijl in Iran mede de steun van Russische missies werd genoten. Ik kan niet ingaan op de wijze, waarop de oorlog tegen het insect tot dusver werd gevoerd. Ik stip slechts aan, dat onder de strijdmiddelen loopgraven, draadversperringen, vlammenwerpers en vliegtuigen een plaats innamen. Belangrijker is evenwel, dat juist in de laatste jaren het vergiftigde lokaas sterk op de voorgrond is getreden, dank zij de bereiding van chemische stoffen, welke in uiterst geringe hoeveelheid insecten doden en welke nochtans voor hogere organismen maar weinig vergiftig zijn. Bij de lokaasmethode vermengt men een of ander voor de sprinkhanen aantrekkelijk voedsel, zoals b.v. vochtige zemelen, met 0,5% ruw hexachloorbenzeen, dat ongeveer 13% van het vergiftige 'gammexaan' bevat. Een ervaren bestrijder slaagt er in met 2,5 kg vergiftigde zemelen, welke dus maar 2 gram van het eigenlijke insecticide bevatten, 5.000.000 sprinkhanen per acre te doden.

Ik stap hiermede af van de beschouwing van de insecten als bela-

gers onze voedselvoorziening, hoewel dit onderwerp nog voor vrijwel onbeperkte uitbreiding vatbaar is. Slechts wil ik nog even het beeld oproepen van de calamiteit, welke Europa zou treffen, indien een verslapte waakzaamheid van de mens er toe zou leiden, dat de gevreesde thans reeds in ons land in hoeveelheden van liters verzamelde Colorado-kever onze aardappeloogst zou vernietigen.

Dan mag ik niet nalaten er aan te herinneren, dat het niet alleen de insecten zijn, welke onze voedingsgewassen bedreigen. Het is algemeen bekend hoe in de jaren 1845 en 1846 in West-Europa hongersnoden heersten, doordat de schimmel *Phytophthora infestans* de aardappeloogst voor een belangrijk deel deed mislukken. Dat deze plaag in latere jaren zich niet meer in gelijke mate heeft doen gelden is slechts te danken aan de krachtige afweermaatregelen van de phytopathologen. Dat ook de meteoroloog bij deze strijd is ingeschakeld zal velen Uwer uit de des zomers per radio uitgezonden berichten wel bekend zijn.

Maar behalve van de insecten en de schimmels is nog melding te maken van een gans andere klasse van levensvormen, welke als belagers van onze voedselvoorziening optreedt. U zijt er ongetwijfeld mee vertrouwd, dat heden ten dage ook voor tal van plantenziekten is vastgesteld, dat zij worden veroorzaakt door virussen, dat zijn smetstoffen van zo geringe afmetingen, dat zij filters passeren, waarvan de poriën zo fijn zijn, dat zij alle met de beste microscopen waar te nemen deeltjes tegenhouden.

Het opmerkelijke bij deze virussen is nu, dat men ze – althans sommige – in zuivere toestand uit de geïnfecteerde planten heeft kunnen afscheiden, waarbij ze als bepaalde chemische individuen, te weten: nucleoproteïnen, zijn gekarakteriseerd. Dit heeft uiteraard de vraag doen rijzen of deze agentia wel aanspraak mogen doen gelden op het praedicaat 'levend', waarvoor anderzijds het vermogen tot vermeerdering in de aangetaste plant weer sterk pleit. Ik laat deze strijdvraag thans rusten en vraag liever Uw aandacht voor het feit, dat de phytopathologen eenstemmig van oordeel zijn, dat het aantal der virusziekten, welke in onze cultuurgewassen optreden, in de laatste jaren sterk is toegenomen en ogenschijnlijk nog steeds stijgt. Nog onlangs heeft de Rector Magnificus der Landbouwhogeschool, Prof. Smit, zich hierover op hartstochtelijke wijze geuit, waarbij hij de gedachte uitwerkte, dat deze vermeerdering van het aantal virussen zou moeten

worden gezien als een reactie van de natuur tegen het misbruik, dat de mens van de aardoppervlakte maakt. Achter dit leger der virussen ontwaren wij kennelijk weer de schim van *homo ignorans*.

Wanneer wij hierbij beseffen, dat reeds thans in vele gevallen uitgebreide maatregelen moeten worden getroffen om het gebruikte plant-materiaal vrij van virus te houden – iets waarop o.m. onze heden ten dage zo belangrijke uitvoer van pootaardappelen grotendeels berust – dan is het duidelijk, dat op dit gebied voor *homo militans* in de toekomst nog een veel zwaardere en omvangrijkere taak is weggelegd.

Maar het buiten-menselijke leven bedreigt de mensheid niet alleen door het afsnijden van de voedseltoevoer. Tal van levensvormen toch, waaronder bacteriën en virussen op de voorgrond treden, hebben de potentie om de mens zelve tot voedingssubstraat te kiezen. Te weinig beseft de leek, dat wanneer in de laatste decennia pandemieën zijn uitgebleven en besmettelijke ziekten dus geen grote bressen in de mensheid hebben geslagen, dit uitsluitend te danken is aan de voortdurende waakzaamheid van de onderling samenwerkende gezondheidsdiensten in de verschillende landen. Deze internationale coöperatie heeft haar hoogste vorm gevonden in de in 1946 in het leven geroepen 'World Health Organization' van de Verenigde Naties, een voortzetting van een overeenkomstige activiteit van de Volkenbond. Juist waar de hedendaagse mens wat betreft de gevaren der besmettelijke ziekten zozeer in slaap is gewiegd, lijkt het niet overbodig hier enkele voorbeelden aan te halen van de plagen, welke in de loop der tijden het voortbestaan der mensheid min of meer ernstig hebben bedreigd.

Ik vermeld dan in de eerste plaats de reeks van pest-epidemieën, welke in de 14de eeuw over Europa en de overige bekende delen der wereld trokken.

Aan het boek van F. Eberson, 'The Microbe's Challenge', ontleen ik, dat naar schatting in Europa alleen al 25.000.000 mensen aan de ziekte stierven en in totaal tennaastenbij 60.000.000, zijnde dit ongeveer één kwart van de gehele wereldbevolking van die tijd. Een goede beschrijving van de symptomen der ziekte, welke de diagnose pest buiten twijfel stelt, danken wij aan Boccaccio, die merkwaardigerwijze zelf in 1348 voor de ziekte vluchtende een vrijwillig gekozen exiel in Florence besteedde om aldaar zijn Decamerone te schrijven.

Het staat thans wel vast, dat de elkaar opvolgende epidemieën Europa vanuit Azië, waar de pest endemisch is, over het nabije Oosten

en Noord-Afrika hebben bereikt. Het is belangwekkend op te merken, dat de mens reeds in die tijden zijn eerste schuchtere pogingen tot verweer deed. In verschillende havensteden ontwaakte het besef, dat de ziekte somtijds blijkbaar per schip werd ingevoerd en in Marseille kwam in 1383 een verordening tot stand, waarbij in verdachte gevallen de bemanning der schepen gehouden was 40 dagen aan boord te blijven alvorens te debarkeren. Aan deze maatregel, welke ook heden ten dage nog een onmisbare strijdmiddel is, zij het dan ook los van de genoemde tijdsduur, danken wij het thans nog gangbare woord *quarantaine*.

Ik zie er van af U een overzicht te geven van de talrijke pest-epidemieën, welke in later eeuwen Europa overvielen. Slechts vermeld ik nog terloops de epidemie van 1663 tot 1668, welke ook ons land teisterde. In eerstgenoemd jaar stierven alleen in Amsterdam 10.000 van de 200.000 inwoners, in het daaropvolgende jaar steeg het aantal sterfgevallen in de hoofdstad zelfs tot ongeveer 24.000.

Eerst in 1894 hebben Kitasato en Yersin onafhankelijk van elkaar aangetoond, dat de pest werd veroorzaakt door een bacterie, thans *Pasteurella pestis* genaamd, terwijl spoedig daarna door verschillende onderzoekers het bewijs werd geleverd, dat de pest primair een ziekte is van ratten en andere knaagdieren, waarbij dan vlooiën verantwoordelijk zijn voor de overgang van dier op mens. Sindsdien is de strijd tegen de pest vóór alles een strijd tegen de rat geworden.

Men zal nu opmerken, dat de pest-epidemieën in Europa toch maar spontaan zijn verdwenen en dit terwijl de mens hierbij toch groten-deels passief is gebleven. Maar dan moet er op worden gewezen, dat in Aziatische landen deze ziekte nog steeds voortgaat op grote schaal slachtoffers te maken. Alleen al in Brits-Indië zijn in de periode 1898–1918 nog meer dan 10.000.000 personen aan de pest gestorven. Men huivert bij de gedachte van hetgeen zich op het zo dicht bevolkte Java zou hebben afgespeeld, indien de Dienst van de Pestbestrijding in de laatste decennia zich niet met zulk een energie had geworpen op het ‘ratproof’-maken van de behuizing der Javaanse bevolking en Otten niet in later jaren 94% van de 2.000.000 bewoners der Preanger prophylactisch had behandeld met het door hem bereide levende vaccin.

Veel opschudding veroorzaakte in 1910 de door een zeer hoge mortaliteit gekenmerkte longenpest-epidemie in Mandsjoerije, waar niet de rat maar de aldaar veel voorkomende, om haar pels zeer gezochte

Siberische marmot (tarbagan) de bron der infectie is gebleken te zijn. Een tweede epidemie, welke tien jaren later uitbrak, werd door de inmiddels tot stand gekomen 'North Manchurian Plague Prevention Service' ondanks de moeilijke omstandigheden, waaronder moest worden gewerkt, met succes bestreden, tengevolge waarvan het aantal slachtoffers veel geringer bleef. Men bedenke voorts, dat ook in deze eeuw zich nog herhaaldelijk sporadische gevallen van pest hebben voorgedaan in verschillende grote havensteden als Glasgow, Sydney, Melbourne, San Francisco e.a., terwijl Van Loghem in de periode van 1913-1920 in een achttal gevallen rattenpest aan boord van schepen in de haven van Amsterdam aantrof. Dat het in geen van deze gevallen tot een epidemische uitbarsting is gekomen mag zeker voor een groot deel aan de effectieve maatregelen van *homo militans* worden toegeschreven.

Als een tweede voorbeeld van een onzichtbare levensvorm, welke nog in recente tijd de mensheid met kans op succes naar het leven heeft gestaan, noem ik de influenza, waarvan de verwekker een virus is gebleken te zijn, dat eerst in de allerlaatste tijd door Stanley is afgezonderd en met het electronen-microscop tot afbeelding is gebracht. Het heeft zich hierbij ontpopt als een nietig bolletje met een diameter van 0,0001 mm. Velen Uwer zullen zich de Spaanse griep-epidemie van 1918, welke ook hier te lande zoveel personen in de kracht van hun leven heeft weggesleept, nog herinneren. Maar niet allen zullen wellicht realiseren, dat dit tragisch gebeuren een onderdeel was van een zich over de ganse aarde uitstreckende pandemie, welke 500.000.000 mensen aantastte en hiervan 15.000.000 doodde. Tijdens het hoogtepunt der ziekte kwam het sterftecijfer overeen met een sterfte van één op iedere 50 personen per maand, een sterftecijfer, dat nog nimmer eerder was voorgekomen. In de Verenigde Staten bezweken in 4 maanden tijds 500.000 personen, een aantal, dat de totale Amerikaanse verliezen in de laatste wereldoorlog merkbaar overschrijdt.

De Amerikaanse geleerde Stanley merkt naar aanleiding van deze cijfers op: 'I have never been able to understand mankind's resigned and complacent acceptance of such a major catastrophe as the 1918 outbreak of influenza. True, the cause of influenza was unknown in 1918, but one would think that such a calamity would result in a great public demand for a concerted effort to discover the cause of the

disease and to develop methods of protection. But this great destruction of human life was accepted almost as ancient peoples accepted natural catastrophes as acts of the gods, and hence beyond the control of man.'

Stanley beschrijft dan hoe eerst ruim 20 jaren later de oorlogsactie er toe leidde, dat het Amerikaanse 'Committee on Medical Research of the Office of Scientific Research and Development' de beschikking kreeg over de middelen voor onderzoeken in grote stijl. Aan deze studiën danken wij niet alleen onze visuele kennismaking met deze zo minuscule gesel der mensheid, maar ook de massale voortkweking daarvan in het laboratorium op kippenembryo's en de omzetting van het aldus verkregen product in een beschermend vaccin. Hoezeer de situatie door het voorkomen van verschillende typen van influenza ook moge worden gecompliceerd, toch aarzelt Stanley niet uit te spreken, dat de mensheid thans over de middelen beschikt om de influenza te verhinderen andermaal als een van de grote vernietigers van menselijk leven op te treden.

Ten bewijze dat ook heden ten dage nog voortdurend gevaren dreigen, wil ik nog even melding maken van de cholera-epidemie in Egypte, waarvan de eerste gevallen op 23 September van het vorige jaar werden geconstateerd. Deze epidemie liet zich aanvankelijk zeer bedenkelijk aanzien en verspreidde zich snel door vrijwel geheel Egypte. Maar *homo militans* sloeg snel toe en in het bijzonder dank zij de vlot verleende steun van de 'World Health Organization' slaagde men er in binnen korte tijd 80% der bevolking te vaccineren, waarna de epidemie snel tot stilstand kwam; na 8 December zijn geen nieuwe gevallen meer gerapporteerd. Dank zij genoemde organisatie werden aan het Egyptische gouvernement ruim 6.500.000 milliliters vaccin op korte termijn toegezonden; 19 verschillende landen, waaronder zowel de Verenigde Staten, als de Soviët Republieken, als ook China en Korea voorkwamen, hadden hiertoe bijdragen geleverd. Dit neemt niet weg, dat de epidemie nog ruim 10.000 slachtoffers heeft gemaakt, welk cijfer evenwel gunstig afsteekt tegen de 34.945 sterfgevallen veroorzaakt door de voorafgaande, uit 1902 daterende, Egyptische cholera-epidemie.

Ik wil thans enige documentatie geven van mijn in de aanvang gedane uitspraak, dat plagen als de zoëven genoemde somtijds op de

ontwikkelingsgeschiedenis van de mensheid onmiskenbare invloed hebben uitgeoefend. Ik ontleen hierbij mijn gegevens aan het zo aantrekkelijke boek van de te vroeg van ons weggenomen Amerikaanse bacterioloog H. Zinsser, welk boek tot titel draagt: 'Rats, Lice and History' en dat door de schrijver nader wordt aangeduid als een biographie van de vlektyphus.

Zinsser geeft in een inleidend hoofdstuk allereerst een pleidooi voor zijn zienswijze, dat uitgebreide, niet meer met zekerheid te identificeren epidemieën in belangrijke mate hebben bijgedragen tot de ondergang van het trotse Romeinse Rijk. Zo hebben ons beschrijvigen bereikt van een epidemie, welke in 165 n. Chr. in het leger van Verus in het Oosten begon en zich na korte tijd vanuit Rome over het gehele gebied 'van Perzië tot de oevers van de Rijn' verspreidde. Orosius meldt, dat tengevolge van de ziekte verschillende steden en dorpen in Italië werden ontruimd en tot ruïnes vervielen. Vast staat, dat deze verschrikking minstens 14 jaren aanhield om daarna te verdwijnen tot negen jaren later een nieuw opblaaien plaats vond, waaromtrent Dio Cassius mededeelt, dat er alleen in Rome dagelijks 2000 doden vielen. Het is begrijpelijk, dat onder deze omstandigheden, waarbij steden werden ontvolkt, de landbouw nagenoeg werd vernietigd en de handel werd stil gelegd, het Romeinse politieke apparaat sterk werd beïnvloed en verschillende militaire campagnes werden afgelast. In het jaar 250 kwam een nieuwe epidemie over Rome met afwijkende symptomen en grote contact-besmettelijkheid, welke zich uiteindelijk uitstreckte van 'Egypte tot Schotland' en welke minstens 15 jaren aanhield. Hiervan getuigt Hieronymus, dat het menselijk ras bijna had opgehouden te bestaan en dat de aarde terugkeerde naar een toestand van woestijnen en wouden. Ik sla nu verschillende kleinere plagen over en vermeld alleen nog de grote pest-epidemie, welke in het midden van de 6de eeuw ten tijde van Keizer Justinianus het Romeinse Rijk in zijn volle omvang trof. Procopius heeft een drastische beschrijving gegeven van de toestand in Byzantium, waar het sterftecijfer tot 10.000 per dag opliep, zodat er geen mogelijkheid meer was om alle lijken te begraven. Veelal werden deze in schepen geladen, welke dan aan de zee werden prijsgegeven. In talrijke Italiaanse provincies bleef de grond onbebouwd en onder de drievoudige druk van pestilentie, hongersnood en de opdringende Lombarden ging het Romeinse Rijk ten onder.

In Zinsser's boek vindt men verder een rijke opsomming van veldtochten en oorlogen, waarvoor doorgaans de aanvoerder der overwinnaars grote roem oogstte, maar die in werkelijkheid door ziekte onder de verslagenen werden beslist. Ik stip er slechts enkele van aan.

In de eerste plaats vermeld ik dan de belangrijke rol, welke de vlektyphus heeft gespeeld bij het beleg van Napels in 1528, toen het restant van het Spaanse leger, dat tevoren triomfantelijk Rome had geplunderd en paus Clemens VII had gevangen genomen, zich hier had verschanst tegen het zegevierend oprukkende Franse leger. Philibert, Prins van Oranje, die de Spanjaarden aanvoerde, schreef aan Karel V, dat de situatie zeer dreigend was en het garnizoen het wel geen maand meer zou kunnen houden. Op de geschiedenis van Europa zou dit van diepgaande invloed zijn geweest: zowel Italië als Clemens VII zouden zeker Frans I met vreugde als bevrijder en beschermer van het geloof hebben aanvaard. Maar inmiddels was onder de belegerende Franse troepen vlektyphus uitgebroken en wel in die mate, dat van de 25.000 man er binnen een maand nog slechts 4.000 over waren. Het gevolg was, dat de belegerden uitbraken en de nog resterende Franse troepen verstrooiden. Karel V had volledig gezegevierd: Italië kwam geheel onder de Spaanse macht en ook Clemens VII gaf zich volledig gewonnen. In 1530 kroonde hij te Bologna Karel tot Keizer van het Romeinse Rijk: het virus van de vlektyphus had het zo gewild.

Zinsser wijst er voorts op hoe de dertigjarige oorlog het best kan worden gekenmerkt als het meest gigantische epidemiologische experiment, waaraan de mensheid ooit is onderworpen. Europa toonde het beeld van een aaneengesloten reeks van uitbarstingen van alle denkbare infectieuze ziekten. Door dit gebied trokken dan 30 jaren legers heen en weer, kleinere benden, vluchtelingen en deserteurs zwierven overal rond. Hongersnood was het gevolg en gehele bevolkingen trokken als horden weg, zoekende naar voedsel en bescherming. En waarheen de mens ook trok, overal volgde hem de ziekte. Onder deze omstandigheden is het duidelijk, dat de uitslag van de strijd niet door de sterkte, de moed of de leiding der strijdende legers, doch door het onzichtbare leven – of indien U dit prefereert: door het toeval – werd beslist. Treffend wordt dit geïllustreerd door de volgende episode. In 1632 stonden Gustaaf Adolf en Wallenstein tegenover elkaar in een strijd om Neurenberg. De vlektyphus bemoeide zich evenwel met het

geval, doodde 18.000 soldaten met het gevolg, dat beide legers alvorens slag te hebben geleverd zich snel terugtrokken om aan de pestilentie te ontsnappen.

Ofschoon het ontstaan van de Republiek Haiti doorgaans aan het genie van Toussaint l'Ouverture wordt toegeschreven, is het virus van de gele koorts daarvoor primair verantwoordelijk. In 1801 zond Napoleon een leger van 25.000 man om de opstand der negers te onderdrukken. Toussaint werd spoedig grondig verslagen en vluchtte in het binnenland; kort daarop brak onder de Franse troepen gele koorts uit tengevolge waarvan niet minder dan 22.000 stierven. Het droevige overschot kon in 1803 slechts evacueren.

Het boek over Napoleon's tocht naar Rusland, dat geschreven werd door Chevalier de Kerckhove, die de expeditie als hooggeplaatst officier van gezondheid meemaakte, laat nauwelijks twijfel, dat de vernietiging van de 'Grande Armée' veeleer aan de vlektyphus en de dysenterie is toe te schrijven dan aan de tegenstand der Russen.

Het in 1813 door Napoleon opnieuw bijeengebrachte leger van 500.000 man verloor in korte tijd door ziekte alleen al 219.000 man. Het schijnt wel zeker, dat Napoleon's macht in Europa veel eerder door ziekte dan door militaire weerstand is gebroken.

Van de Krim-oorlog kan niet worden getuigd, dat hier ziekte de balans naar één kant deed doorslaan, maar daar hier voor het eerst betrouwbare statistieken zijn aangelegd kan met zekerheid worden vastgesteld, dat bij alle partijen de verliezen aan vlektyphus, cholera en andere ziekten, die, welke aan het gevecht waren te wijten, vele malen overtroffen.

Nu zal men wellicht opmerken, dat deze zaken grotendeels betrekking hebben op een ver achter ons liggend verleden en dat het heden ten dage hiermede nogal los pleegt te lopen. Men zal dan denken aan de beide wereldoorlogen, welke nog zo vers in het geheugen liggen en waaraan men niet vóór alles een beeld van ziekte op grote schaal bejaart.

Maar dan moet worden beseft, dat, wanneer in de huidige tijd *homo militans* optrekt om zijn onderlinge geschillen te beslechten, hij er ten volle rekening mede houdt, dat hij een groot deel van zijn activiteit moet beleggen in de strijd tegen de alomtegenwoordige onzichtbare derde. Alleen op deze wijze is het te verklaren, dat in de eerste wereldoorlog de strijdende machten – dank zij een energieke ontluizings-

politiek – er in zijn geslaagd het Westfront vrij van vlektyphus te houden. Maar als wij Zinsser mogen geloven, had de vlektyphus een niet onbelangrijk aandeel in de geallieerde overwinning, doordat de vrijwel onmiddellijk in Servië opgestoken epidemie, welke in 6 maanden 150.000 slachtoffers maakte, het Oostenrijkse leger er van terughield het land binnen te trekken wat in zijn consequenties op de overige Balkanlanden waarschijnlijk de centrale mogendheden buitengewoon ten goede zou zijn gekomen.

Wat de tweede wereldoorlog betreft, is het nog te vroeg om de balans op te maken aangaande de invloed van het onzichtbare leven op de gang van zaken. Maar vele van onze landgenoten, die naar Duitsland zijn weggevoerd, hebben ervaren dat in het bijzonder in het laatste chaotische jaar de vlektyphus zijn kop weer opstak, zij het dan ook dat *homo militans* er in slaagde de rampen te localiseren.

Het zou mij verheugen, indien mijn auditorium aan de hier gegeven enigszins macabere voorbeelden, welke overigens nog met talrijke andere zouden zijn uit te breiden, de overtuiging zou kunnen ontleenen, dat inderdaad niet zelden de loop der geschiedenis door de onzichtbare levende wereld is bepaald en dat, indien de mensheid voor de toekomst haar aardse lot van dergelijke invloeden vrij wenst te houden, voortdurende actie van *homo militans* geboden blijft.

Men zou nu evenwel kunnen opmerken, dat het voorafgaande alleen betrekking heeft op perioden van oorlog en chaos, maar dat men in meer normale tijden althans in West-Europa toch veilig leefde achter de nu eenmaal door onze gezondheidsdiensten opgeworpen hygiënische barrières. Maar dan moet hierop worden geantwoord, dat enkele onzichtbare levensvormen er toch nog altijd met vrij veel succes in slaagden deze barrières te passeren. Dit geldt b.v. voor *Mycobacterium tuberculosis*, de verwekker der tuberculose, niet zelden als 'Volksvijand No. 1' afgeschilderd. Talrijk zijn ook hier te lande nog degenen, die aan deze wrede ziekte te gronde gaan, dan wel door haar worden geïnactiveerd. Hoewel het einddoel nog verre van bereikt is, mag gelukkig worden geconstateerd, dat *homo militans* ook hier sinds geruime tijd actief is en zijn laatste strijdmiddel moge hier wel even worden gememoreerd.

Gedachtig aan het gezegde, dat men dieven het beste met dieven vangt, en onder de indruk van de successen, welke met het U allen

bekende penicilline zijn behaald, heeft men er naar gestreefd de strijdmiddelen, waarmede verschillende microben elkaar onderling beoorlogen, ook voor dit geval toe te passen. Door isolatie van de aan *Mycobacterium tuberculosis* antagonistische *Actinomyces griseus* werd het mogelijk het chemische principe, waarvan deze actinomyceet zich in zijn strijd bedient en dat de naam van streptomycine verkreeg, af te scheiden. Ofschoon het streptomycine voor het menselijke lichaam niet altijd geheel onschadelijk is gebleken te zijn, heeft men er toch bij bepaalde vormen van tuberculose verrassend goede resultaten mee bereikt.

Voor één punt moet ik eveneens nog Uw aandacht vragen. Men zou kunnen menen, dat de microbioloog thans toch wel zal beschikken over een volledige catalogus van alle onzichtbare levensvormen, welke de mens rechtstreeks kunnen bedreigen, zodat verrassingen in de verdere strijd zijn uit te sluiten. Maar dan moet hierop worden geantwoord, dat deze mening onjuist is en dat in de latere jaren ziekten zijn opgedoken, waarvan men op goede gronden aanneemt, dat zij in vroegere tijdperken niet voorkwamen.

Gelukkig kan dadelijk hiertegenover worden gesteld, dat er ook sterke aanwijzingen zijn, dat andere ziekteverwekkers, welke in achter ons liggende eeuwen grote ravages hebben aangericht, inmiddels zijn uitgestorven. Als voorbeeld van dit laatste kan worden genoemd de 'English sweating sickness', die men voor het eerst beschreven vindt in 1485, maar waarvan men na 1552 nimmer meer iets heeft vernomen.

De eerste epidemie brak uit na de slag van Bosworth en leidde tot een ontbinding van de zegevierende troepen onder Hendrik VII, wiens kroning dan ook moest worden uitgesteld. De ziekte breidde zich over geheel Engeland uit; de mortaliteit was zeer hoog: slechts één op de 100 aangetaste personen placht aan de dood te ontsnappen. Nadien braken in 1507, 1518, 1529 en 1551 nog nieuwe epidemieën uit, welke zich ten dele ook tot het continent uitstrekten, hierna heeft men nimmer meer van een ziekte met de beschreven symptomen gehoord. Iets soortgelijks doet zich voor met een enigszins verwante, maar toch duidelijk af te grenzen ziekte, welke bekend staat als 'la suette des Picards', omdat zij het eerst in Picardië is opgetreden.

Na dit uitstapje over verdwenen ziekten keer ik nog even terug naar het onderwerp van de 'nieuwe' ziekten, een gezichtspunt dat een

waarschuwing inhoudt om de toekomstige strijd met het onzichtbare leven niet te onderschatten.

In dit verband moge eerst iets worden opgemerkt over de oorsprong van het parasitisme. Het blijkt, dat er maar weinig ziekteverwekkers zijn, die niet een onmiskenbare verwantschap vertonen met heden ten dage nog in de vrije natuur aangetroffen, z.g. saprophytische vormen. Onder deze omstandigheden moeten we het optreden van parasieten wel hieraan toeschrijven, dat sommige dezer vrijlevende vormen, die als alle leven aan geleidelijke veranderingen onderhevig zijn, er in de loop der tijden in zijn geslaagd de natuurlijke weerstand te doorbreken, welke ieder levend celcomplex biedt tegen indringing van een soortvreemd leven. Er is alle aanleiding om aan te nemen, dat dit proces een aanvang heeft genomen in een ver verleden in de geschiedenis van het leven op aarde. De palaeontologen hebben zelfs bij zeer oude fossielen, teruggaande tot het Perm (200.000.000 jaren geleden) duidelijk aanwijzingen van infectieziekten geconstateerd en sommige onderzoekers zijn zelfs geneigd het uitsterven van talrijke diersoorten in praehistorische tijden aan dergelijke ziekten te wijten.

Het lijkt nu geenszins uitgesloten, dat een dergelijke overgang van saprophytische tot parasitaire leefwijze ook heden ten dage zich nog wel eens bij bepaalde vormen zal kunnen voltrekken. Deze mogelijkheid wordt verhoogd door het feit, dat de microbioloog in latere jaren er in is geslaagd met behulp van straling kunstmatige mutaties bij tal van microben teweeg te brengen, terwijl het alleszins denkbaar is, dat onder natuurlijke voorwaarden de cosmische straling soortgelijke effecten bewerkt.

Maar voorts is er rekening mede te houden, dat bij ononderbroken overdracht van gastheer op gastheer de eenvormigheid van het milieu veranderingen in eigenschappen van de parasiet kan bewerken, welke zowel ten goede als ten kwade kunnen strekken.

Terwijl een eerste invasie doorgaans tot een heftige reactie zal leiden, waarbij hetzij de gastheer hetzij de indringer acuut bezwijkt, zal een zekere aanpassing primair een chronisch verlopende afwijking bij de gastheer veroorzaken, waarop later zelfs een wederzijds samenleven, waarbij de gastheer geen symptomen van letsel vertoont, zal kunnen volgen. Hier kan b.v. worden gewezen op de bekende spirochaetose van vele muizen, terwijl ons medelid Swellengrebel een sensationeel voorbeeld uit de menselijke pathologie vond, toen hij vast-

stelde, dat in het bijzonder de jeugd-generatie van de Bosnegers in Suriname veelal in sterke mate met virulente malariaparasieten was besmet zonder daarvan ogenschijnlijk schade te ondervinden.

Intussen kan een dergelijke aanpassing ook tot geheel andere consequenties leiden. In tal van gevallen is gebleken, dat de passage door een soortvreemde gastheer de eigenschappen van virussen ingrijpend wijzigt. Zo bewerkt het virus van de gele koorts in apen symptomen, welke veel overeenkomst met die der menselijke ziekte vertonen. In muizen geënt bewerkt hetzelfde virus daarentegen een vorm van encephalitis, welke in serie op muizen kan worden overgedragen. Ent men hierop dit virus weer in apen dan treden geen gele koortsverschijnselen op, maar het virus behoudt zijn neurotroop karakter.

Duidelijk blijkt hieruit, dat wij ieder ogenblik met nieuwe ziektebeelden ook van de mens kunnen worden geconfronteerd, zonder dat dit op een 'de novo'-creatie van nieuwe parasitaire vormen behoeft te worden teruggevoerd.

Hiernaast bestaat er voor de mens nog een andere bron voor nieuwe ziekten, namelijk het voor de eerste maal in contact komen met in het dierenrijk reeds ingeburgerde vormen van parasitisme, waarbij steeds de mogelijkheid van een overgang op de menselijke gastheer aanwezig is. Een typisch voorbeeld van een aldus ontstane nieuwe virusziekte is de in 1879 door Ritter voor het eerst beschreven psittacose, waarbij kakatoe's of parkieten ongetwijfeld als reservoirdier van het zo infectieuze, voor de mens sterk pathogene virus optreden.

Een tweede geval van een vermoedelijk eerst in recente tijd opgetreden ziekte werd het eerst in 1910 beschreven als een ziekte van de 'groundsquirrel' in Tulare County in Californië. De verwekker van deze ziekte bleek een tot die tijd onbekende bacteriesoort, welke enigszins verwant is aan de pest-bacterie maar daarvan toch duidelijk verschilt. Deze bacterie heeft sedert de naam van *Pasteurella tularensis* verkregen. Negen jaren later brak in een geheel ander deel der Verenigde Staten, namelijk in Utah, een ziekte uit onder lieden, die hadden deelgenomen aan een jacht op wilde konijnen, waarbij een deel van de buit was gestroopt en voor voedsel was meegenomen. Opmerkelijkerwijze werd in dit geval zowel bij de patiënten als bij de wilde konijnen *P. tularensis* aangetroffen. Sedert is de tularemia, zoals men de ziekte heeft genoemd, niet alleen op vele plaatsen in Amerika,

maar ook in Noorwegen, Zweden, Rusland en Japan veelal in epidemische vorm opgetreden.

Het zijn nu juist de verwekkers van beide laatstgenoemde nieuwe ziekten, waartegen het massale verweer nog in de kinderschoenen staat, die in het bijzonder ook zijn betrokken bij de preventieve onderzoeken over experimentele infectieverspreiding, welke tijdens de oorlog in Camp Detrick in de Verenigde Staten zijn ondernomen.

Hoezeer wij vurig moeten hopen, dat de mensheid de schande van de bacteriënoorlog bespaard zal blijven, moge het ons Hollanders nederig stemmen, dat het een landgenoot was, die waarschijnlijk als eerste deze vorm van oorlog heeft gepropageerd. Het was de twee eeuwen geleden te Haarlem als zoon van een medicus geboren Pieter van Woensel, die in zijn in 1790 verschenen 'Reis-Aantekeningen' rapporteert over zijn ervaringen opgedaan tijdens een vijfjarig verblijf in Turkije, aan welk land hij kennelijk zijn liefde heeft verpand. Bezorgd voor de bedreiging van Constantinopel door de Russen ontwikkelt hij in zijn boek een methode van afweer gebaseerd op de mogelijkheid tot kunstmatige verspreiding van de pest onder de bevolking van het aanvallende land. Men weet niet, wat in Van Woensel's uiteenzetting meer treft: de grote schroom waarmee hij zijn denkbeeld ontvouwt, dan wel het haast lyrische commentaar, dat hij uiteindelijk aan de efficiëntie van het door hem gepropageerde verweermiddel wijdt.

Sprak ik in het voorafgaande enkel over de invloed, welke van de ziekteverwekkende vertegenwoordigers van het onzichtbare leven op de mensheid en haar daden is uitgegaan, ook andere op zichzelf geheel onschuldige microben hebben zich in dit opzicht niet onbetuigd gelaten. Een treffend voorbeeld hiervan vinden wij in de z.g. wonderbacterie, welke heden ten dage de wetenschappelijke benaming *Serratia marcescens* draagt. Deze zeer algemeen verspreide bacteriesoort heeft de eigenschap een rode kleurstof te produceren, tengevolge waarvan een plaatselijke ontwikkeling der bacterie leidt tot de vorming van een rode massa, welke een onmiskenbare gelijkenis met een bloedvlek vertoont.

In het bijzonder voedingsmiddelen als brood, polenta (maispap) e.d., welke rijk aan zetmeel zijn, vormen voor de ontwikkeling der bacterie een gunstig substraat.

Het kan niet verwonderen, dat in een tijd, waarin men met de na-

tuurlijke oorsprong van een dergelijke 'bloedvorming' niet bekend was, het bijgeloof hieraan verstrekkende conclusies verbond. Omtrent de consequenties hiervan zijn in oude kronieken talrijke mededelingen te vinden, waarvan men een overzicht kan aantreffen in de verhandelingen van Ehrenberg, van Scheurlen en van Harrison. Zeer uiteenlopend was de reactie van de menselijke ziel op het optreden van 'bloed', in het bijzonder ook wanneer het verschijnsel zich bij het eucharistisch brood voordeed. Soms werd dit als 'het wonder' aanvaard en leidde het slechts tot verhoogde devotie der gelovigen. Onder meer legt Raphael's beroemde schilderij getiteld 'Het wonder van Bolsena' hiervan getuigenis af. Niet minder zelden echter werd het verschijnsel anders geïnterpreteerd en werd 'de schuld' gezocht onder de ongelovigen, in het bijzonder ook onder de Joodse bevolking. Bij herhaling hebben deze bij honderden hiervoor op de brandstapel moeten boeten en dit heeft Scheurlen verlokt tot de uitspraak, dat de zo onschuldige wonderbacterie meer doden op haar geweten heeft dan menige voor de mens pathogene microbe. Een in 1819 min of meer epidemisch opgetreden rode verkleuring van uiteenlopende voedingsstoffen in verschillende dorpen in de provincie Padua leidde tot een systematisch onderzoek door verschillende Italiaanse geleerden, van wie Bizio de eerste was, die overtuigend aantoonde, dat de roodkleuring het gevolg was van de ontwikkeling van een plantaardig organisme van zeer geringe afmetingen. Sedert is het optreden der wonderbacterie praktisch aan de sfeer van het bijgeloof onttrokken.

De geschiedenis van de wonderbacterie leert, dat van een microbe ook een niet te onderschatten psychische beïnvloeding van de mens kan uitgaan. Dat dit ook voor de ziekteverwekkende kiemen geldt en dat deze dus naast de bewerking van de eerder besproken massale effecten ook op de individuele menselijke psyche een blijvende stempel kunnen drukken, zal voor ieder aannemelijk zijn, die kennis heeft genomen van het voortreffelijke boek van Albert Camus 'La Peste', dat terecht onder de Franse literaire productie uit de naoorlogse tijd in sterke mate de aandacht heeft getrokken. Bij deze uitspraak dienen wij in het oog te houden, dat wij hier het terrein van de fictie betreden en dat er van de zijde der medici en hygiënisten zeker gegronde critiek op Camus' schildering der pestepidemie en haar gevolgen kan worden uitgeoefend. Aan de literaire waarde van het boek doet dit evenwel niet af en dit temeer niet, omdat de schrijver ten volle de

mogelijkheid open laat, dat wij zijn beschrijving van het leven in een door pest geteisterde, van de buitenwereld afgesloten stad moeten zien als een transpositie van het leven in een onder terreur zuchtend bezet gebied. Dit neemt evenwel niet weg, dat de reactie van de verschillende door Camus geschetste karakters op een voortdurende, niet af te wenden levensbedreiging dermate aanvaardbaar lijkt, dat wij alle reden hebben om te besluiten, dat in werkelijke epidemieën zich soortgelijke psychische processen zullen hebben afgespeeld.

Aan het einde van mijn uiteenzetting gekomen laat zich niet langer verhelen, dat aan de keuze van de titel van mijn voordracht een element van misleiding niet vreemd is. Ik moge dan in het voorafgaande gelegenheid hebben gevonden te doen uitkomen, dat in de ontwikkelingsgeschiedenis van de mensheid een *homo militans* in onafgebroken strijd met buiten-menselijke levensvormen in toenemende mate een rol speelt, maar over dit mensentype en over de drijfveren, welke hem in zijn strijd bezielen, sprak ik U niet.

Hier zou een diepgaande analyse van de levensbeschrijving van hen, die bij deze strijd in de voorste gelederen staan of hebben gestaan, belangrijke gezichtspunten kunnen openen. Ik zal hiernaar niet streven en beperk mij in dit opzicht tot enkele losse opmerkingen.

Zeker is, dat wij *homo militans* in zeer verschillende gedaanten kunnen aantreffen.

Daar is in de eerste plaats de man, die gedreven door ergernis over de schade, welke een bepaalde levensvorm de mensheid berokkent, zich de strijd daartegen tot levensdoel stelt.

Een typisch voorbeeld hiervan is b.v. de Amerikaanse specialist J. L. Nicholes, die in zijn recent boek getiteld 'Vandals of the Night' verslag heeft uitgebracht over zijn levenslange strijd tegen de rat en die er zich op kan beroemen niet minder dan 25.000.000 individuen van deze vijand der mensheid te hebben opgeruimd. Hierbij moge nog terloops worden opgemerkt, dat deze veteraan allerminst optimistisch oordeelt over de afloop van de strijd. Op grond van zijn ervaringen is hij dermate onder de indruk gekomen van het grote aanpassingsvermogen van zijn vijand, dat zijn conclusie luidt, dat er op zijn minst een kans is, dat de rat de mens zal overleven.

Verwant aan deze fanatieke specialisten, maar toch daarvan afwijkend, zijn nu de strijders, die zich niet tegen bepaalde vijanden rich-

ten, maar die zich zeer in het algemeen tot taak stellen de voedselvoorziening der mensheid tegen alle denkbare belagers veilig te stellen. Zij worden aangevoerd door veldheren, die over uitgebreide generale staven en doeltreffende organisaties tot uitvoering van de noodzakelijk geoordeelde maatregelen beschikken.

De aanvoerders zijn veelal phytopathologen of economische entomologen; de in de aanvang van mijn voordracht genoemde entomoloog Howard levert hiervoor een typerend voorbeeld op. Maar onder de comilitanten treft men ook aan nuchtere statistici, geographen, sociographen, meteorologen en vele andere werkers, die wellicht de belagers der voedselproductie en de daaraan toegebrachte schade nimmer hebben aanschouwd. Tenslotte is er dan het leger der uitvoerders, waaronder we enerzijds weer scherpzinnige biologen en chemici, maar ook landbouwers, controleurs en vliegtuigpiloten opmerken.

Hiernaast ontmoeten wij *homo militans* dan in de gedaante van hen, die in verwoede strijd zijn gewikkeld met die onzichtbare levensvormen, welke rechtstreeks de gezondheid en het voortbestaan der mensheid in ernstige mate bedreigen. Ook hier heeft de strijd reeds lang tot de instelling van machtige, zich over de gehele wereld uitstreckende organisaties geleid, waarvan vele strijders deel uitmaken, aan wie de eigenlijke medische professie ten enenmale vreemd is. Maar ook voor de leiders in de strijd, de 'men against death', zoals De Kruif ze in één van zijn boeken op geslaagde wijze heeft gekarakteriseerd, geldt weer, dat zeer uiteenlopende motieven hen tot strijder hebben gemaakt. In de eerste plaats is er dan de geleerde 'à outrance', de wetenschappelijk bezetene, die alles wil doorgronden en die daarin ook betrekt, waarom de mens somtijds door onzichtbare krachten wordt geveld. Men denke hierbij aan een figuur als Pasteur en ik geloof, dat ik aan de onschatbare verdiensten van deze grote weldoener der mensheid geen afbreuk doe, wanneer ik opmerk, dat bij dit type van strijders de gedachte aan therapie en preventie toch steeds op het tweede plan staat.

Hiertegenover staan dan degenen, die in hun strijd worden voortgedreven door opstandigheid tegen of erbarmen met het lijden van hun medemens, door het idee van niet te mogen berusten in diens ondergang. Semmelweis, de overwinnaar van de kraamvrouwenkoorts, is een typische representant van deze variant van *homo militans*. Even-

eens onder de minder prominente strijders bevinden zich velen, die hoewel minder gepassioneerd zich nochtans inzetten gedreven door een bewust streven om de lijdende mensheid te dienen. Zeker geldt dit voor die uitzonderlijke lieden, die zich in bepaalde gevallen beschikbaar hebben gesteld om als menselijke proefdieren dienst te doen bij de studie van levensgevaarlijke ziekten en waarvan de Amerikaanse militairen, die in 1900 op Cuba Reed in staat stelden het raadsel der gele koorts te doorgronden, het meest bekende, hoewel geenszins alleen staande, voorbeeld vormen.

Tenslotte mogen naast deze mensen, die zich bewust offerden 'for the cause of humanity and the interest of science' niet vergeten worden de velen voor wie waarschijnlijk van toepassing is, wat Camus in zijn zoëven genoemd boek één van zijn geïmproviseerde pestbestrijders laat zeggen. Gepeild naar de motieven voor zijn zo riskante medewerking, wordt het antwoord aarzelend samengevat in de simpele woorden 'par honnêteté'.

Ik besef, dat ik met deze enkele opmerkingen allerminst een passend beeld heb ontworpen van *homo militans*, zoals deze zich heden ten dage voordoet. Het zou voor mij evenwel reeds een grote voldoening zijn, indien ik er in zou zijn geslaagd mijn auditorium er van te doordringen, dat het voor de *homines militantes* dezer aarde de hoogste tijd is zich niet langer uit te putten in een roemloze onderlinge strijd, maar zich te wijden aan de dringender en tegelijk schoner taak: het voortbestaan der mensheid tegen haar vele belagers te verzekeren.

In dit geval zult Gij in de menselijke familiekring naast *homo sapiens*, *homo faber* en niet te vergeten *homo ludens* wel een blijvende plaats willen inruimen voor *homo militans*!

MICROBIAL METABOLISM AND ITS INDUSTRIAL IMPLICATIONS

THE liberation of the Microbiological Panel of your Society from the restriction of being merely a part of the Food Group, and its promotion to the status of an independent Society Group, mark a wider recognition of the significance of the microbe world for chemical industry. The invited lecturer might, therefore, be expected to give a convincing justification of this change in the organization of the Society.

The difficulty of fulfilling this task properly is enhanced by the mixed character of the audience. It seems probable that this will partly be composed of captains of chemical industry who scarcely ever will have given microbes a thought, and partly of representatives of the several hundreds of members of the former Microbiological Panel, which means that amongst this group there will be masters of industrial microbiology for whom a plea for the industrial importance of microbes would just mean 'carrying coal to Newcastle'. Fortunately, recent events have made this time-honoured phrase lose its aptness to denote absurdity, and this has encouraged an attempt to satisfy both parties.

The active part which the Microbiological Panel of the Food Group has taken for several years in the proceedings of your Society testifies that the food industries at least are aware that mankind is surrounded by a world of invisible life which continuously lies in wait and which at often unexpected moments may manifest its presence by an outburst of proliferation.

This will generally lead to most undesirable effects; it is, therefore, quite comprehensible that amongst food manufacturers microbes have a bad reputation. It is, however, perhaps not always realized that the socially justified hoarding of food which is the main characteristic of our food industries, and which is indissolubly connected with the present structure of human society, is contrary to the laws of nature. Seen from the standpoint of the microbes any preservation of surplus

food for future human consumption is an unlawful, although mainly temporary, interference with their rights.

Anyhow, we all know that this situation has led to the development of a prosperous food preservation industry in which nowadays the battle against the microbe world is directed along scientific lines. However, as is demonstrated by the 'flat sours', the 'hard swells' and the cases of 'sulphide spoilage' which from time to time occur in the canning industry, this battle does not always end victoriously. A thorough study of the micro-organisms responsible for these various types of spoilage is, therefore, a point of primary importance. It is interesting to note that even after several decades of intensive research sometimes new types of spoilage and new microbial culprits may still be discovered. I can refer here to a recent investigation made by my collaborator Verhoeven who found that in nitrate-cured canned ham the swelling of the cans is not due as usual to an evolution of hydrogen and carbon dioxide in the can, but mainly to the evolution of a mixture of nitrogen and nitrous oxide by some denitrifying bacterium.

The circumstance that we meet 'laughing gas' here is particularly noteworthy when we think of the remarkable physiological effects of this gas on man. In this historic place, a short digression may perhaps be permissible. Through a historical study made by my late colleague Cohen of Utrecht I am acquainted with the fact that thanks to Davy's enthusiasm nitrous oxide has played a prominent rôle in the early history of the Royal Institution and that exactly one and a half centuries ago these revered surroundings were the scene of remarkable demonstrations of its physiological action. The fact that nitrous oxide can be a product of microbial metabolism or, in other words, can originate in a living cell, therefore appears in a special light.

A characteristic example of an industry in which an invasion of microbes can easily be expected is offered by the sugar industry. In this connexion the interesting observations made in this country by De Whalley and Scarr on the microflora which sometimes interferes with sugar refining should be mentioned, but it seems to me that a more systematic investigation of the rôle which micro-organisms play in the sugar industry as a whole still remains to be done.

It had already been recognized some 60 years ago that the clogging of pipelines in this industry which often led to serious difficulties in manufacturing was due to the proliferation of a capsule-forming strep-

tococcus earlier described as *Leuconostoc mesenteroides*. However, ten years ago my collaborator Perquin found that this so-called 'dextran'-formation could also be caused by a quite different rod-shaped bacterium which, remarkably enough, had been described in 1897 by Marshall Ward as one of the components of the 'ginger-beer plant'. The same has lately been found in England by Scarr.

Another interesting observation was recently made by Verhoeven who found that the nitrite sometimes present in molasses and which may give rise to serious complications if the molasses are to be applied in the fermentation industry, owes its origin to nitrate-reducing bacteria accumulating in the crude juice almost immediately after it leaves the diffusion battery.

It is generally known that troublesome deviations from the normal course of manufacture, owing to an invasion by microbes, are not at all restricted to the food industries. It has been realized for some time that several organic raw materials which on the whole are unsuitable as human or animal food are readily accepted as such by special types of micro-organisms. This may lead to various undesirable industrial implications; I need only cite the mildewing of cotton fabrics as an example. The important work performed in the Shirley Institute has made it possible to devise effective counter-measures. In the mildewing of fabrics and related materials, the microbial nature of the trouble has been evident almost from the beginning. This cannot be said of slime formation in paper mills which may seriously interfere with production, and which in the past has often led to expensive interruptions in the process, caused by the need for cleaning. Since in later years it was shown that bacteria are nearly always responsible for the slime production, this trouble has almost completely been overcome by adding biocides like pentachlorophenol or organic mercury compounds to the pulp mass.

We all know, too, that an organic construction material like wood is, under certain conditions, liable to disastrous attacks by special representatives of the microbe world, the more or less ubiquitous wood-destroying fungi. The response of mankind has been a wood preservation industry which in later years has owed much to the extensive research work done in various laboratories, amongst which the Forest Products Research Laboratory at Princes Risborough holds first place.

It therefore seems justified to conclude that all industrial leaders

dealing with the more common organic materials would do well to keep a constant careful watch for objectionable manifestations of microbial life in their factories. I could imagine that some of my hearers would heave sighs of relief because their industrial products do not belong to this vulnerable class, so that they can go on living in a blissful ignorance of what is going on in the microbe world. However, I feel it my duty to disturb this illusion also by putting forward the thesis that only very few industrialists will escape encounters with the invisible life. These may be 'brief encounters', but we all know that these too may have dire consequences. A few examples may suffice.

I mentioned the vulnerability of wood as a construction material, and it seems easy, at first, to avoid the use of this material by having recourse to materials like stone or concrete. But investigations of Payne and co-workers have made it highly probable that the stone walls of many historic buildings in England, as for instance those of Westminster Hall, are subject to microbial attack; according to the well-known Russian microbiologist Issatschenko even concrete structures in contact with water may well be corroded by the growth of a special acid-producing microflora at the surface.

The important work done here in London during the war by Smith and his co-workers is well known. This aimed to combat the deterioration of military equipment in a tropical climate. For the manufacturers of leather belts, and especially for those of optical instruments and radio sets, it must have been a revelation to find that they had to take into account the results of microbiological research.

One might think that at least the heavy industry would be in the happy situation of being able to ignore microbial life, but this hope, too, should be abandoned. For the mineral oil industry ZoBell has in recent years collected ample evidence that, under conditions sometimes realized in industry, hydrocarbons can also fall a prey to microbes. He mentions a case in which a pronounced decrease in the octane rating of aviation spirit stored over water was due to bacteria accumulating in the water phase and preferentially attacking the branched chain hydrocarbons in the spirit.

A somewhat analogous case is the following. A machine tool factory seems to be an ideal place to escape interference from micro-organisms. But an investigation made by Lee and Chandler has shown that the oil-water emulsions used as lubricants and cooling agents in the

cutting and grinding of metals are often subject to heavy bacterial growth. This growth is apparently correlated with an attack on the naphthenic acids used as emulsifying agents, thus rendering the cutting compound unfit for further use.

Anyone who beholds the mighty plant of a modern coke industry in which high temperatures and high gas pressures seem to dominate the scene will tend to think this industry to be impervious to microbial attack. Yet I know of an instance in which the extinguishing of an oven battery has been seriously taken into consideration, because of the clogging of the scrubbers in which the coal gas is washed with water: this clogging was directly connected with an almost incredibly rapid and profuse growth of bacteria in the water in contact with the gas under a pressure of 13 atmospheres.

Perhaps still more startling is the experience in a modern nitrogen fixing plant. Here the acid-fast steel cooler in which nitric acid was condensed showed serious signs of corrosion, even leading to perforation, after a relatively short period of use. The management of the factory was initially inclined to doubt the acid fastness of the steel, but on second consideration there appeared to be reason to suspect the tiny bugs which had accumulated in the cooling water on the other side of the steel wall. In this respect I refer to the investigations of von Wolzogen Kühr in Holland, of Thaysen, Bunker and Butlin in England, and of Starkey in the U.S.A. which have thrown a clear light on the active part played by sulphate-reducing bacteria in the anaerobic corrosion of steel and cast-iron tubes.

These examples should suffice to show how microbes may interfere with industry. It seems more important to analyze why this interference leads so often to undesirable effects in human proceedings. It then soon becomes clear that in most cases it is not the presence of the microbes as such that is the cause of our troubles, but the fact that the maintenance and proliferation of a microbial population is accompanied by chemical conversions. In other words, we have to consider microbes as co-practitioners of our craft, and it is evident that the leader of a chemical industry cannot tolerate 'illegal' chemistry in his factory. Obviously the first command for any action is 'know thine enemy', and one of the first things to be attempted should be a survey of the various chemical conversions brought about by micro-organisms, or in other words a survey of microbial metabolism.

Metabolic activity is a common characteristic of all forms of life, but to the physiologist who is conversant with the metabolism of either animals or higher plants the results of the investigations on microbial metabolism must be more or less bewildering. The following exposé may justify this statement.

It is a general experience in all metabolic studies that part of the components of the food which enter a living cell is excreted again in the surrounding medium after having undergone a chemical conversion. It is usual to designate this part of metabolism as 'catabolism' or 'dissimilation', in contrast to those chemical conversions of food components which lead to the building-up of cell constituents, and which are summed up in the terms 'anabolism' or 'assimilation'. It is obvious that the so-called dissimilatory processes can only be important for the cell from the point of view of energy supply. In the animal kingdom these energy-yielding processes are on the whole of one and the same type; broadly speaking they can be characterized as the slow combustion of carbohydrates, fats, and amino-acids to carbon dioxide, water and ammonia. This so-called respiration process is also encountered in the green plants, but here in all cases the primary energy-yielding process is restricted to the photochemical conversion of carbon dioxide and water to a carbohydrate.

The situation in the microbe world is fundamentally different; here we encounter a diversity of dissimilatory processes which is most impressive, because it tends to show that Nature has not neglected any potential source of chemical energy on earth as a basis for sustaining life. Without aiming at completeness, I should like to give some documentation of this almost overwhelming diversity in microbial dissimilation.

First consider the system glucose-oxygen which in higher organisms as a rule is converted into carbon dioxide and water. A study of the way in which different micro-organisms behave towards the system in question has shown that certain species also bring about a complete oxidation of the glucose, but that – at least under certain conditions – in other species quite different conversions of the glucose occur. Table I gives a survey of various ways of oxidative dissimilation of glucose as encountered in the microbe world.

A second remarkable point is that in the bacterial kingdom numer-

TABLE I

Various types of oxidative glucose dissimilation by micro-organisms

1. $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$
carbon dioxide
 2. $C_6H_{12}O_6 + 5O_2 \rightarrow 2HOOC \cdot COOH + 2CO_2 + 4H_2O$
oxalic acid
 3. $C_6H_{12}O_6 + 1\frac{1}{2}O_2 \rightarrow HOOC \cdot CH_2 \cdot \overset{\overset{||}{CH_2}}{C} \cdot COOH + CO_2 + 3H_2O$
itaconic acid
 4. $C_6H_{12}O_6 + 1\frac{1}{2}O_2 \rightarrow HOOC \cdot CH_2 \cdot \overset{\overset{COOH}{|}}{COH} \cdot CH_2 \cdot COOH + 2H_2O$
citric acid
 5. $C_6H_{12}O_6 + O_2 \rightarrow CH_2OH \cdot \overset{\overset{5\text{-ketogluconic acid}}{CO}}{CO} \cdot CHOH \cdot CHOH \cdot CHOH \cdot COOH + H_2O$
 6. $C_6H_{12}O_6 + \frac{1}{2}O_2 \rightarrow CH_2OH \cdot CHOH \cdot CHOH \cdot CHOH \cdot CHOH \cdot COOH$
gluconic acid
 7. $C_6H_{12}O_6 + \frac{1}{2}O_2 \rightarrow CH_2OH \cdot \underset{\underset{\text{kojic acid}}{C}}{C} = \underset{\underset{O}{|}}{CH} - CO - COH = CH + 3H_2O$
-

ous species are not at all confined to the use of glucose or related carbohydrates as a respiratory substrate. On the contrary it has been established that representatives of very different groups of organic compounds can be used as the sole source of carbon for special types of aerobic bacteria, which implies that these bacteria can satisfy their energy demand from an oxidation of the compounds in question. Amongst these compounds are fatty acids, dicarboxylic acids, hydroxy-, keto- and amino-acids, but also aliphatic amines, various aromatic compounds, sterols, hydrocarbons etc. Table II gives some idea of the surprising diversity of substrates which can be used by one and the same bacterial species.

The bacterial world still has several other surprises for the student of comparative physiology. To the genius of Winogradsky we owe the knowledge that representatives of various bacterial groups can thrive in the complete absence of all organic compounds, provided that carbon dioxide is supplied together with some oxidizable inorganic compound. Various types of such autotrophic bacteria have successively been discovered: the so-called nitrifying bacteria which oxidize either ammonia or nitrite, the sulphur bacteria which oxidize hydrogen sulphide, elementary sulphur and some simple sulphur com-

TABLE II

Compounds which can act as sole source of carbon in the development of *Pseudomonas putida* (Flügge) L. and N. (after Den Dooren De Jong)

Fatty acids (saturated)	Alcohols	Amino-acids
Acetic acid	Methyl alcohol	Glycine
Propionic acid	Ethyl alcohol	Sarcosine
Butyric acid	Propyl alcohol	Betaine
<i>iso</i> Butyric acid	<i>iso</i> Butyl alcohol	Acetyl glycine
Valerianic acid	Octyl alcohol	Hippuric acid
Caproic acid	Decyl alcohol	Phenyl glycine
Heptylic acid	α -Propylene glycol	α - and β -Alanine
Caprylic acid	Trimethylene glycol	Phenylalanine
Nonylic acid	2,3-Butylene glycol	Tyrosine
Caprinic acid	Glycerol	Benzoylalanine
		Histidine
Fatty acids (unsaturated)	Carbohydrates and derivatives	δ -Amino-valerianic acid
α -Crotonic acid		α -Amino- <i>iso</i> -valerianic acid
Undecylenic acid	Glucose	Leucine acid
Oleic acid	Fructose	<i>iso</i> Leucine
	Mannose	Aspartic acid
Hydroxy- and keto-acids	Gluconic acid	Glutamic acid
Lactic acid	Saccharic acid	
β -Hydroxy-butyric acid	Mucic acid	Amides
Pyruvic acid		Propionamide
	Amines	Butyramide
Di- and tri-basic acids	Butylamine	<i>iso</i> Butyramide
Malonic acid	<i>iso</i> Butylamine	Succinamide
Succinic acid	Amylamine	Asparagine
Glutaric acid	Aethanolamine	Creatine
Fumaric acid	Cadaverine	Arginine
Aconitic acid	Benzylamine	Allantoine
Malic acid	Histamine	Uric acid
Citric acid		
		Aromatic compounds
Substituted acids		Benzoic acid
Phenylacetic acid		<i>p</i> -Hydroxybenzoic acid
α - and β -Bromo-propionic acid		
Bromo-succinic acid		

pounds to sulphate, and finally iron bacteria which in all probability oxidize not only ferrous ions but also metallic iron (Table III).

TABLE III

Dissimilation processes of some autotrophic micro-organisms

1.	$\text{NH}_3 + 1\frac{1}{2}\text{O}_2 \rightarrow \text{HNO}_2 + \text{H}_2\text{O}$
2.	$\text{HNO}_2 + 1\frac{1}{2}\text{O}_2 \rightarrow \text{HNO}_3$
3.	$\text{H}_2\text{S} + 1\frac{1}{2}\text{O}_2 \rightarrow \text{S} + \text{H}_2\text{O}$
4.	$\text{S} + 1\frac{1}{2}\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4$
5.	$\text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O} + 2\text{O}_2 \rightarrow \text{Na}_2\text{SO}_4 + \text{H}_2\text{SO}_4$
6.	$4\text{FeCO}_3 + \text{O}_2 + 6\text{H}_2\text{O} \rightarrow 4\text{Fe}(\text{OH})_3 + 4\text{CO}_2$
7.	$\text{H}_2 + 1\frac{1}{2}\text{O}_2 \rightarrow \text{H}_2\text{O}$

The biggest surprise of all, however, is that the microbe world contains exceptions to the rule which holds for higher organisms and with which we all are familiar, namely that the presence of free oxygen is an essential condition for the maintenance of life. Since the classical studies of Pasteur we are acquainted with the existence of micro-organisms which can thrive in the absence of oxygen and for which, in certain cases, this gas even has a harmful effect on the activities and proliferation. We all know that Pasteur at once felt the need for another energy-yielding process as a substitute for respiration, and that he did not hesitate to indicate the sugar fermentation process as such.

Alcoholic fermentation is by far the best known of these sugar fermentation processes, but gradually several other types of anaerobic sugar dissimilation have been found to be the energy-yielding processes in certain bacterial groups (Table IV).

These processes are usually designated by the main product formed out of the sugar, as lactic acid, propionic acid, butyric acid, butanol, butanediol and other fermentations.

Even a superficial acquaintance with fermentation processes will suffice to show that such a process presents a peculiar type of chemistry: the number of final products can be large, whilst the quantities of the various products do not have a stoichiometrical relationship; moreover, they vary considerably with the external conditions. This so ap-

parently puzzling situation has been unravelled to the extent that it has become clear that we are dealing with mixed fermentations, or in other words that one part of the substrate molecule undergoes a conversion which differs from that to which a second part is subjected. This obviously explains both the absence of stoichiometrical relationships and the dependence of the results on external conditions. We must, therefore, conclude that bacterial fermentations as a rule comprise a number of elementary processes; some of the more important elementary processes occurring in sugar fermentations have been collected in Table IV.

TABLE IV

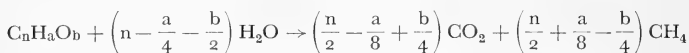
Various types of fermentative glucose dissimilation by micro-organisms
Elementary processes

1. $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\underset{\text{ethyl alcohol}}{\text{CH}_3 \cdot \text{CH}_2\text{OH}} + 2\text{CO}_2$
2. $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\underset{\text{lactic acid}}{\text{CH}_3 \cdot \text{CHOH} \cdot \text{COOH}}$
3. $\text{I}^{1/2}\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\underset{\text{propionic acid}}{\text{CH}_3 \cdot \text{CH}_2 \cdot \text{COOH}} + \underset{\text{acetic acid}}{\text{CH}_3 \cdot \text{COOH}} + \text{CO}_2 + \text{H}_2\text{O}$
4. $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \underset{2,3\text{-butanediol}}{\text{CH}_3 \cdot \text{CHOH} \cdot \text{CHOH} \cdot \text{CH}_3} + 2\text{CO}_2 + \text{H}_2$
5. $3\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\underset{2,3\text{-butanediol}}{\text{CH}_3 \cdot \text{CHOH} \cdot \text{CHOH} \cdot \text{CH}_3} + 2\underset{\text{glycerol}}{\text{CH}_2\text{OH} \cdot \text{CHOH} \cdot \text{CH}_2\text{OH}} + 4\text{CO}_2$
6. $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \underset{\text{butyric acid}}{\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}} + 2\text{CO}_2 + 2\text{H}_2$
7. $\text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{+\text{H}_2\text{O}} \underset{\text{acetone}}{\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_3} + 3\text{CO}_2 + 4\text{H}_2$
8. $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 3\underset{\text{acetic acid}}{\text{CH}_3 \cdot \text{COOH}}$

N.B. Most bacterial fermentations comprise two or more elementary processes.

The separate elementary fermentation processes also present difficult riddles to the chemical mind. The only clear fact is that they show a common characteristic, *viz.*, that certain of the compounds formed are in a reduced state as compared with the substrate, whilst other compounds are oxidized. This is nowhere better shown than in the so-called methane fermentation. Buswell has shown that his crude cultures of methane bacteria succeeded in converting the most diverse

organic compounds almost quantitatively into carbon dioxide and methane. This occurred with such an utter independence of the nature of the substrate that this fermentation could justifiably be summarized in the following general equation:



It would take too long to dwell upon the progress made with regard to our insight into the mechanism of the various fermentation processes. I need only remark that nowadays we know that these gross elementary processes in reality consist of long chains of primary, as a rule reversible, reactions, each of which is promoted by a specific catalyst. The well-known scheme of alcoholic fermentation may be given here by way of example (Table V).

The most remarkable feature of the scheme as a whole is undoubtedly that the primary reactions evidently belong to two reaction types: we are dealing either with the transference of a phosphate group from one molecule to a second one, or with a coupled dehydrogenation and hydrogenation. Another point worth noticing is that the fermentation of one molecule of glucose is indissolubly connected with the formation of two molecules of adenosine triphosphate (ATP) out of two molecules of adenosine diphosphate (ADP). This implies the coupling of the sugar fermentation with the stoichiometric consumption of a second compound.

I shall return to this point later, and wish to remark first that all that has become known about the other sugar fermentation processes agrees with the idea that these too can be reduced to chains of transphosphorylations and transhydrogenations. The same has now been found to hold for the chemistry of the normal respiration process in which glucose is oxidized to carbon dioxide and water (Table VI).

Here it is generally accepted nowadays that the initial conversions which the glucose molecule undergoes are the same as in alcoholic fermentation. However, the pyruvic acid in a complex reaction involving a dehydrogenation is coupled with oxalacetic acid, and in doing so enters the Krebs or tricarboxylic cycle, where four more dehydrogenations occur, and at the end of which oxalacetic acid is regenerated.

It should be remarked by the way that the respiratory chain is also

TABLE V

Alcoholic fermentation

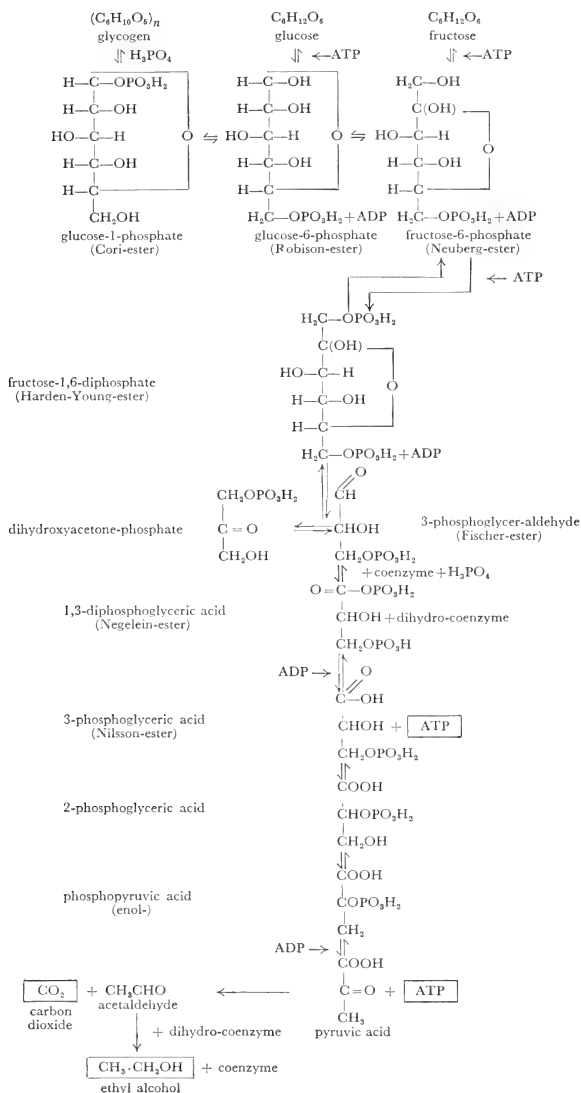
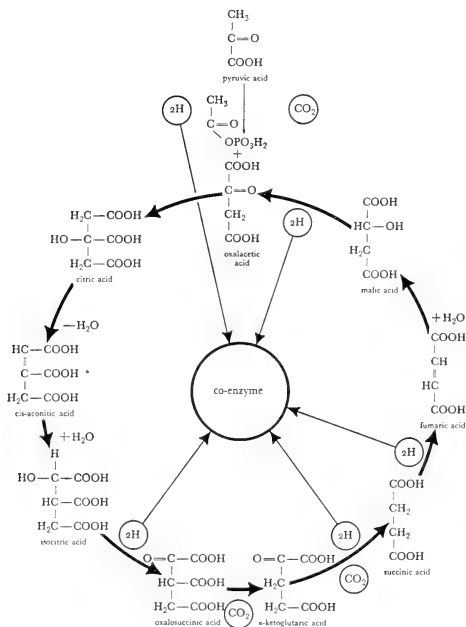


TABLE VI

Tricarboxylic acid cycle



coupled with an uptake of inorganic phosphate leading to an ample formation of ATP; according to Ochoa, not less than 32 molecules of ATP are formed in the combustion of one molecule of glucose.

All the hydrogen removed in the dehydrogenations passes a long chain of respiratory catalysts at the end of which oxygen evidently must act as the final acceptor. This last conclusion is of special im-

portance, since it means that in both respiration and fermentation the reaction

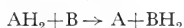


plays an essential role, and that the difference between the two is restricted to the fact that in fermentation the molecule B is organic and derived from the substrate itself, whilst in respiration the molecule which ultimately acts as B is free oxygen.

This throws a clear light on a series of dissimilatory processes which for a long time have occupied a separate position in microbiology. I refer to the processes of nitrate and sulphate reduction, and the process to which I have earlier referred as methane fermentation. For – as indicated in Table VII – there is now every reason to consider these processes as reactions in which nitrate, sulphate and carbonate act as the final hydrogen acceptor B of the general equation given, and in doing so can be reduced to the ultimate hydrogenation stages of nitrogen, sulphur and carbon, which elements thus share the fate of oxygen in respiration.

TABLE VII

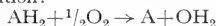
The principle of transhydrogenation as the essence of dissimilation processes



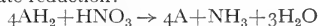
Fermentation:



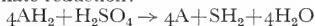
Respiration:



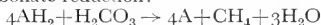
Nitrate reduction:



Sulphate reduction:



Carbonate reduction:



This is another very impressive aspect of the great adaptability of microbial life to potential sources of chemical energy and one is inclined to see this as the result of an evolutionary trend. For germs

which initially were confined to aerobic conditions it must have been a constant temptation to use these reducible ions as a substitute for oxygen at those spots, where this gas was lacking.

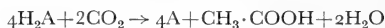
For the denitrifying bacteria the situation is, indeed, that they can thrive either with oxygen or with nitrate, which means that these primarily aerobic organisms can eventually extend their zone of life to those nitrate-containing soil layers in which the air does not enter. The cases of sulphate- and carbonate-reducing bacteria must apparently be seen as a further step in this development, since these bacteria are strictly anaerobic, which implies that free oxygen is no longer a suitable hydrogen acceptor for them.

There are a few more types of dissimilatory reactions which I should like to bring to your attention. My fellow-countryman Wieringa discovered in 1936 an anaerobic bacterium which had the remarkable ability of converting a mixture of carbon dioxide and hydrogen into acetic acid. This means that the hydrogen acceptor function of carbon dioxide is not restricted to its conversion into methane, but under

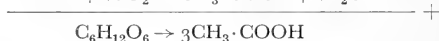
TABLE VIII

Survey of fermentation depending on the formation of acetic acid out of carbon dioxide

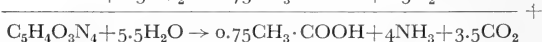
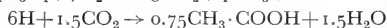
General equation:



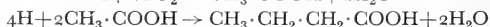
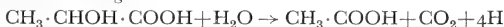
Clostridium acetium:



Clostridium acidu-wici:

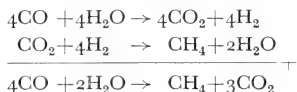


Butyribacterium rettgeri:



certain conditions may proceed less far, thus quite unexpectedly giving rise to the formation of the new C-C bond as present in acetic acid (Table VIII). The American investigator Barker has since shown that this type of carbon dioxide hydrogenation is encountered in several other dissimilation processes. Amongst others, he found that this holds in the acetic acid fermentation of glucose discovered by Fontaine and co-workers in which in principle a glucose molecule is converted into three molecules of acetic acid. This yield will incur the jealousy of the vinegar manufacturers, who, passing the intermediary step of alcoholic fermentation, can never get more than two molecules out of one molecule of glucose.

Finally, I should like to draw your attention to the curious fermentation in which carbon monoxide acts as a substrate. In my laboratory, Schnellen brought definite proof that certain methane-producing bacteria could bring about the following startling reactions:



In view of the highly poisonous character of carbon monoxide for so many forms of life, it is certainly a remarkable biochemical 'stunt' that *Methanosarcina barkeri* in an aqueous medium in contact with a gas phase containing 100 per cent carbon monoxide will convert this gas into a mixture of carbon dioxide and methane!

In illuminating-gas plants, partly depending on production of water gas, this conversion may well occur when gas holders of the wet type are used.

The examples given should have sufficed to establish the conviction that microbial life in its protean variety is constantly lying in wait for chances to exploit potential sources of chemical energy of the most diverse type. Adding to this the surprising ubiquity of a great number of germs which apparently are widely transported through the air from a soil teeming with life, it is easily understood that the most divergent industries are liable to microbial invasions which may lead to definitely troublesome effects.

It is, therefore, not surprising that this continuous struggle of man against microbial life has given rise to industries in which the defence against this menace has become the main purpose. I have already

mentioned the various industries which aim at food preservation, as well as those devoted to the preservation of wood. In addition, I should remark that the industrial production of different types of germicides has nowadays assumed considerable proportions.

Until now I have merely stressed the necessity for the industrialist to be quite vigilant in order to prevent 'illegal' chemistry in his factory. However, the remarkable chemical achievements of micro-organisms have quite naturally already led to the attempt to domesticate special types in order to employ them for the bulk production of certain desirable products.

Many obstacles had to be overcome in order to safeguard the domestic microbes against the competitive actions of their wild confrères, but considerable success in this has gradually been achieved.

The industrial production of ethyl alcohol by the fermentation of sugars is, of course, the oldest example. Although nowadays there is severe competition from synthetic processes, more than half of the 187,500,000 gallons produced in the U.S.A. in 1949 was obtained by fermentation. With lactic acid the situation is even more favourable, in so far as the industrial production of this compound is still completely based on the fermentation process, and the same holds for a minor product like butyric acid.

Apart from these classical fermentations which have developed from a more or less empirical start, other dissimilatory processes have been successfully applied in more recent times for the bulk production of certain valuable organic compounds. We need only mention the industrial production of *n*-butyl alcohol and acetone by fermentation of corn mash or molasses, and the production of citric acid from the latter source. Both industries have attained considerable dimensions, the annual production in the U.S. alone amounting to about 70,000 tons of butyl alcohol, and to 15,000 tons of citric acid. Another fermentation process which has found practical application in Holland, albeit on a small scale only, is the production of 2,3-butanediol.

From the examples given earlier it will be clear that there are still many other microbial dissimilatory processes which could be considered for industrial application, and which from time to time have received attention in this respect. I can refer to the production of kojic acid and itaconic acid by moulds and to the recent suggestion made by your compatriot Butlin to apply the dissimilation process of the

sulphate-reducing bacteria in order to cope with the menace of the world sulphur shortage.

The question which should occupy us now is, what are the prospects in a not too far distant future for a bulk production of relatively simple organic compounds by micro-organisms?

I regret that my answer can only be that in my opinion these prospects are not bright.

Without decrying the great merits of many industrial microbiologists, it appears difficult to escape the conclusion that the enormous progress which synthetic organic chemistry has made in later years almost inevitably leads to some cheap process for the synthesis of any fermentation product which has proved to be a commercial success. As a rule the microbiologist will be unable to compete with synthetic chemistry which starts from the 'cheap carbon' in fossil raw materials like coal, mineral oil or natural gas. In this respect the continuously declining part which fermentation products have in the total production of ethyl alcohol, butyl alcohol and acetone may be deemed symptomatic.

At first sight there seems to be only one consolation. The earth's reservoir of fossil fuel is apt to be exhausted in a not too far-away future. At that moment mankind will again have to depend on the products of the recent green vegetable kingdom also for its organic syntheses. In the conversion of the carbohydrates of the world-crops into useful chemicals the industrial microbiologist of the future may again achieve new triumphs. This consideration leads to the more or less paradoxical conclusion that industrial microbiology may be a useful science, but that it may as well be 'mothballed' for one or two centuries!

Fortunately the situation is quite different. Until now I have only dealt with those manifestations of microbial metabolism which may lead to a wholesale production of comparatively simple organic compounds. Yet the true force of the microbe world is to be found in the production of characteristic vital compounds often of complicated structure, or in the production of inextricable and refined mixtures of compounds with a definite physiological activity.

The latter part of this statement will not need much documentation. It seems quite possible that, in time, the chemist may succeed in producing a tolerable gin by using ethyl alcohol from coke gas and adding some cleverly mixed synthetic essence. But I do not think that we shall

ever meet an organic chemist who is so ambitious that he will endeavour to synthesize a French 'vin de château' or one of the many dairy products which owe their merits to their characteristic flavour, such as Roquefort cheese. Here, there will always be a task for the microbiologist, namely to direct microbial life into the required paths.

However, still more important than the preparation of these complex luxuries will be the conscious preparation of certain valuable products of microbial metabolism, of such particular structure that their purely chemical synthesis cannot be achieved, at least not economically. Various factors may be responsible for the latter restriction. In some cases this condition may already be fulfilled, although the required compound has a simple configuration. This holds, for instance, when one is dealing with compounds containing an asymmetrical carbon atom; synthesis will always produce the two stereoisomers in equal quantities, whilst owing to the asymmetrical character of the catalysts active in the microbial cell one of the isomers is often formed either preferentially or exclusively. This difference may be of decisive importance. The purely chemical preparation of lactic acid has made much progress in recent years. In as far as the lactic acid is prepared as a constituent of products for human consumption there is every reason to demand that the acid should be the physiological form, *i.e.*, l(+)-lactic acid. The product of organic synthesis will never meet this requirement; on the other hand, the industrial microbiologist will be able to prepare the right form by a deliberate selection of the bacterial strain to be applied in the fermentation (Table IX).

TABLE IX

Stereoisomers of lactic acid produced by representatives of different genera of lactic acid bacteria

<i>Thermobacterium</i> d (—) or i	<i>Microbacterium</i> l (+)
<i>Streptobacterium</i> l (+) or i	<i>Streptococcus</i> l (+)
<i>Betabacterium</i> i	<i>Betacoccus</i> l (+) or i
<i>Pediococcus</i> i	

A somewhat similar case occurs in the preparation of a compound like 2,3-butanediol which is known to exist in three configurations: laevo-, dextro-, meso-.

TABLE X

Survey of fermentations leading to the formation of 2,3-butanediol

Aerobacter aerogenes (chiefly meso, besides dextro)

Aerobacillus polymyxa (laevo)

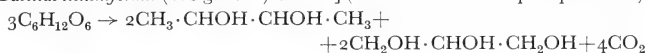


Serratia marcescens (chiefly meso, besides dextro)



Bacillus subtilis Ford-strain

[= *Bacillus licheniformis* (Weigmann) Gibson] (meso and laevo in equal quantities)



As is shown in Table X the configuration of the diol formed depends on the type of bacteria used in the fermentation. In Canada the use as 'antifreeze' of the laevo-compound, as formed practically exclusively in the fermentation of sugar or starch by *Aerobacillus polymyxa*, has been seriously considered. In contrast, the meso-form of the diol has been shown to be completely unsuitable for the purpose.

In such special cases, therefore, even the bulk production of microbial dissimilatory products, may be able to compete successfully with synthetic processes.

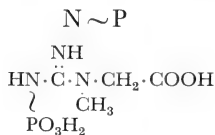
New vistas for industrial microbiology are revealed when we realize that the foregoing discussion has been confined to the dissimilatory processes, but that the true greatness of microbial metabolism manifests itself in the assimilatory processes, *i.e.*, in those processes which are the basis of the proliferation of the micro-organism. Evidently, part of the food components are converted into cell constituents in their almost endless diversity. Although as a rule assimilation generally ranks far below dissimilation from a quantitative point of view, compensation may often be found in the qualitative value of many assimilation products for mankind. It seems difficult to overrate the remarkableness of these chemical reactions which together constitute assimilation, and the word super-chemistry may well be coined for them.

Although the riddles of this 'super-chemistry' are still far from being solved, great progress has been made during the last decade.

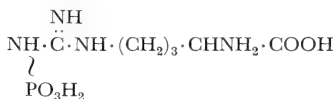
The main barrier to a better understanding of assimilatory chem-

TABLE XI

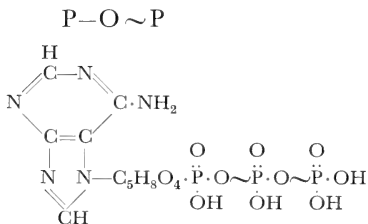
Energy-rich phosphate bonds



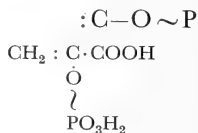
creatine phosphate



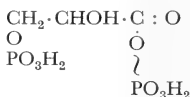
arginine phosphate



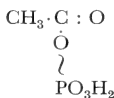
adenosine tri-phosphate



phospho-enol pyruvic acid



phosphoglyceryl phosphate



acetyl phosphate

istry has always been that in many cases the conversion of part of the substrate into some assimilatory product seemed to require reactions in which the free energy of the system showed an increase. This applied for instance in the production of fat from glucose. In order to explain this, it was rightly emphasized that the conversion was always coupled with a second conversion marked by a significant decrease in free energy, as for instance the respiration process. But the way in which the cell succeeded in putting the energy of one chemical reaction at the disposal of a second one has for a very long time remained obscure.

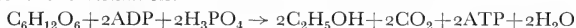
Lipmann's discovery of the so-called energy-rich phosphate bond has more or less solved this problem. This investigator has definitely shown that several organic phosphate compounds which are quite

common in living cells are characterized by the fact that the splitting off of the phosphate group is accompanied by an unusually strong decrease in free energy. This implies that the phosphorylated compound has a much higher energy content than the phosphate-free moiety, and that therefore this moiety may, simultaneously with the dephosphorylation, enter into reactions which otherwise would be impossible (Table XI). Amongst cell constituents with such energy-rich phosphate bonds I mention here adenosine triphosphate – as far as the pyrophosphate bonds are concerned – the acetyl phosphate discovered by Lipmann, and two compounds we have met in the scheme of the sugar dissimilation, namely 1,3-diphosphoglyceric acid and phospho-enol pyruvic acid. We have seen that the intermediate formation of these latter compounds in sugar fermentation leads to the formation of ATP out of ADP, ultimately implying that the integrate formulation of the process of alcoholic fermentation is given by the equation reproduced in Table XII.

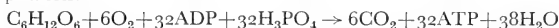
TABLE XII

Integrate formulation of the processes of:

(a) Alcoholic fermentation:



(b) Respiration:



ADP = adenosine diphosphate

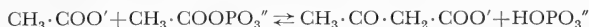
ATP = adenosine triphosphate

By analogy there is reason to accept the integrate formulation of the respiration process as given in the same table, although this formulation bears, as we have already remarked, an almost fully empirical character, the reactions leading to this abundant ATP formation having been only partly revealed.

In any case there is nowadays no doubt that ATP should be considered as the material link between the energy-yielding dissimilatory processes and the energy-demanding assimilatory processes. Through its two energy-rich phosphate bonds ATP gives rise to the formation of other compounds with similar bonds, which compounds then act

teins of *Torulopsis utilis* after this yeast had been grown in a medium containing an ammonium salt and sodium acetate in which the two carbon atoms had been labelled with the C^{13} and C^{14} isotopes respectively. Their results agree with the hypothesis that the keto-acids occurring in the tricarboxylic acid-cycle are either directly or indirectly involved in the synthesis of several of the amino-acids.

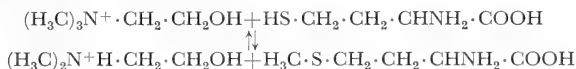
As for the synthesis of fatty acids, we have learnt from the important investigations of Lipmann and of Barker that a transfer of the acetyl group present in acetyl phosphate plays an important rôle. Several arguments favour the primary reaction:



This type of reaction which repeats itself in the synthesis of higher fatty acids can aptly be designated as transacetylation.

Finally, we owe to the work of Challenger – and as far as animal metabolism is concerned especially to that of Du Vigneaud – the knowledge that in the synthesis of several other biologically important compounds a transfer of methyl groups, or in other words a transmethylation, is an essential step.

A typical reaction of this type is the reaction between choline and homocysteine which leads to the formation of dimethylamino-ethanol and methionine according to the equation:



We may conclude from the foregoing that, although the riddles of the superchemistry of assimilation are still far from being solved, all the available evidence supports the idea that we are dealing here with chains of primary reactions which all belong to one of the following five categories: transhydrogenation, transphosphorylation, transamination, transacetylation and transmethylation.

Carbohydrates, fats and proteins are the main quantitative constituents of the microbial cell, and this makes one realize that micro-organisms might at some time be used as human food or animal fodder, the more so because the amazing synthetic capacity of many microbes permits them to build up these pillars of animal nutrition, and especially the proteins, in a medium containing nothing but a single organic compound together with some inorganic salts. Because of this, mi-

crobes are potential competitors for the green plants which are the basis of our food supply. Successful efforts have been made in this country by Thaysen to arrive at an economically warranted production of food yeast, and if at present the odds are still in favour of other foodstuffs it does not seem unlikely that in a not too far future the question: 'Yeast or meat, Madam?' will be quite commonplace in restaurants.

In comparison with yeast and similar micro-organisms the green plants of our world crops are favoured by their photo-synthetical capacity which allows them to renounce organic nutrients, and to depend on atmospheric carbon dioxide as their sole source of carbon. For this reason it seems of great significance that the arsenal of the industrial microbiologist has recently been reinforced by the addition of some unicellular green algae, such as *Chlorella vulgaris*, which are also equipped with a photo-synthetic apparatus. The future will teach us whether a mass production of unicellular algae will prove to be a remunerative proposition; the practical difficulties involved seem, indeed, to be considerable. The fact that under favourable conditions *Chlorella* is potentially able to produce 4 tons of dry material per acre in 8 days against only 1.5 tons of corn per acre in 90 days, gives a ready explanation for the great interest of our American colleagues.

Although at present the conditions for a wholesale production of microbial cell material for the use of human or animal nutrition are not yet fulfilled, nevertheless several microbes are now being cultivated in order to obtain some particularly valuable cell constituent which, owing to its more or less complex configuration, is not easily accessible by chemical synthesis. This holds unconditionally for various enzyme preparations which nowadays are produced on a considerable scale, especially in the U.S.A., both from moulds and from bacteria. Microbial preparations which bring about a hydrolysis of starch, pectin or proteins have found wide application in several industries.

Another microbial product which on account of its complex configuration does not need to fear competition from organic synthesis has recently come to the fore. I refer to the bacterial polysaccharide dextran, already mentioned in connexion with the sugar industry. Its formation by the lactic acid bacteria is remarkable, because this glucose polymer is only produced from saccharose, and not at al

from glucose. It is the great merit of Stacey to have first considered its industrial production and the industry both in England and Sweden has recently increased greatly in importance, owing to the discovery made by Ingelman and Gronwall that partially hydrolyzed dextran is a valuable substitute for blood plasma.

The production of certain vitamins offers another example. Vitamin B_2 or riboflavin, both in the pure form and as riboflavin concentrates, is being used increasingly for the fortification of fodder and in the U.S.A. of human food also. By far the greater part of this riboflavin is being produced by growing the fungus *Eremothecium ashbyi* under suitable conditions. This organism can be made to synthesize riboflavin so much in excess of its own needs that the vitamin crystallizes in the cell and is partly excreted into the medium. It is worth mentioning that for the isolation or concentration of the riboflavin from the fermented mash, use is often made of a second micro-organism which converts the vitamin into its practically insoluble hydrogenation product.

Also, for the production of Vitamin B_{12} , either pure or as a concentrate, and of the closely related animal protein factor, certain actinomycetes and bacteria are being cultivated on an increasing scale. Since in this production a yield of 1 mg per litre is apparently still considered to be quite satisfactory, there seems to be ample room for improvement in production methods.

However, several of these products could also be manufactured from raw materials of animal or vegetable origin. It is important that it has become increasingly clear, of late, that certain micro-organisms produce highly specific compounds, albeit in small quantities only; moreover, some of these products are able to render eminent services to mankind. I refer to the antibiotics, the large-scale production of which has given a tremendous impetus to the development of industrial microbiology in the last decade.

The amazingly strong biological action of certain microbial metabolic products had been established long before the word antibiotic was coined. Almost from the beginning of microbiology it has been known that the fatal influence which certain bacteria exert on man or animal is due to the excretion by the bacteria of some product which in an extremely low concentration is toxic to the host. The isolation by a group of American investigators of some of these bacte-

rial toxins in a pure and crystalline state is certainly one of the most remarkable biochemical achievements attained in recent years. The establishment that the toxin of *Clostridium botulinum* Type A is a protein of high molecular weight, containing amongst others all ten amino-acids essential for animal nutrition, is extremely surprising in view of the almost incredible toxicity of the product. The fact that one microgram of such a protein has the potency for killing 20,000 mice is one of the most startling physiological effects known, and is still awaiting explanation.

The quantities of antibiotics needed to attain a definite bacteriostatic or bactericidal effect, though small, are large when compared with the quantities of toxins needed for their action.

It is not, therefore, the low doses required for a noticeable activity of the antibiotics which makes them so remarkable and such mighty tools in human therapy, but their amazing specificity. It is not so surprising that some hundredths of a microgram of penicillin per millilitre medium inhibit the development of many bacteria, as the fact that this antibiotic even in much higher concentration does not have any harmful effect on many other bacterial species or on the body cells of man and animal.

Some time ago the General Assembly of the United Nations specified and condemned the crime of 'genocide' or 'race discrimination in murdering'; it is very obvious that in the microbe world either such laws do not exist, or that microbes are far from being law-abiding creatures.

The discovery of the various antibiotics like penicillin, streptomycin, chloramphenicol, aureomycin, terramycin and bacitracine has created a mighty microbiological industry in which either moulds, actinomycetes or bacteria are cultivated on a huge scale. If one tells a layman that the world production of penicillin for 1950 – and also that of streptomycin – was about 150 tons he will probably not be much impressed. But as soon as one can make him realize that for each of these products 30,000,000 gallons of culture media have been prepared, inoculated and harvested, a new light will be thrown on this tremendous achievement of our industrial microbiologists.

Of course, even this statement does not do justice to the accomplishments of the microbiologist. Before any industrial production of an antibiotic, thousands of micro-organisms may have been isolated from

nature; each one is tested for its antagonistic properties and if these are satisfactory, the optimal conditions for production of the antagonistic principle will have been determined. At this stage the biochemist may take over the job of devising suitable methods for the isolation of the active principle, and if successful will attempt to establish its chemical nature. Then the pharmacologist will have to make the necessary animal experiments in order to decide on the degree of toxicity. If his reports are also favourable, production on a somewhat larger scale will be necessary to provide the material needed for a thorough clinical test. And in the quite exceptional case that the new antibiotic passes all these stages, a new branch of microbiological industry may develop.

How should we judge the prospects for a further development of the antibiotic industry? It seems that a bright future still lies ahead. For a documentation of this statement it is instructive to glance at the chemical formulae of the three established antibiotics of which the chemical constitution has been elucidated, to wit: penicillin, streptomycin and chloramphenicol (Table XIII).

Here we have three molecules which were all quite new to science when their configurations were determined. Before their discovery, even the boldest organic chemist would hardly have considered their synthesis. If he had, nothing but a miracle could have induced him to test the therapeutic value of his preparations, none of the structures suggesting anything of their beneficial specific action.

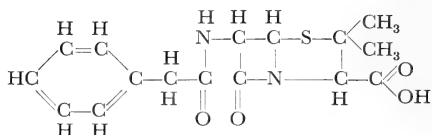
It is true that each molecule considered separately has its remarkable features. For penicillin, both the thiazolidine ring and the *beta*-lactam ring are characteristic and – as far as I know – the latter has never before been encountered in naturally occurring compounds.

As for the formula of streptomycin, it can most aptly be described as the product of an organic chemist just before his admittance to a lunatic asylum. The structures of each of the three moieties of the molecule are equally irrational and the mere idea that nature has thought fit to combine these elements to form a molecule with remarkably specific action tends to depress the most modest amongst biologists.

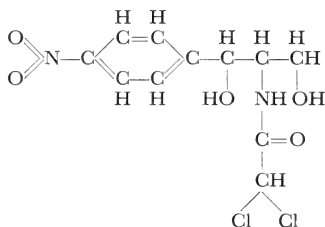
Finally, there remains the miracle of chloramphenicol. It is true that its structure is much more simple, so much so that it has fallen a relatively easy prey to the organic chemist. But here we encounter two

TABLE XIII

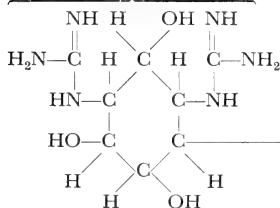
Penicillin G



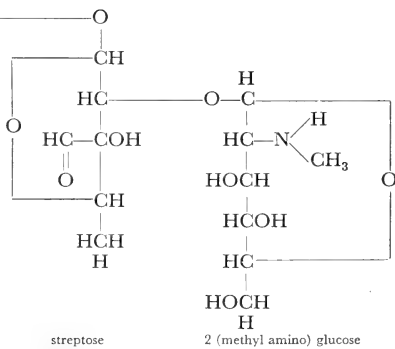
Chloramphenicol



Streptidine



Streptobiosamine



1,3-diguanidino-2,4, 5,6-tetrahydroxy cyclohexane

streptose

2 (methyl amino) glucose

Streptomycin

features which are quite unusual for a metabolic product. Although the merit of the first discovery of a mould product containing non-ionic chlorine falls to Raistrick and his group working at the London School of Hygiene, further examples of such compounds have remained rare, and organic chemists, taking into account their own achievements, will not be able to suppress their admiration for *Streptomyces venezuelae* which succeeds in chlorinating some organic compound in an aqueous medium at room temperature. Moreover, scarcely recovered from this surprise, one discovers the nitro-group in the molecule, a new feature for a naturally-occurring compound, and especially striking because of the marked activity of other nitro-compounds in physiology.

However, the most surprising point of all is that three molecules which are so utterly different should be equally effective in inhibiting the growth of a great number of bacterial species, whilst at the same time showing striking differences in their behaviour towards other bacteria.

Thus, one decade of antibiotic research has led to the discovery of a series of economically important microbial metabolic products never dreamed of in our philosophy until that time. New terms will probably be added to this series, and aureomycin, neomycin and terramycin are already there to corroborate this.

May we not hope that in a not too far distant future a further penetration into the secrets of microbial metabolism will lead to the discovery of a penicillin substitute fit for oral administration, a streptomycin substitute without side-effects and which does not induce resistance in *Mycobacterium tuberculosis* and a chloramphenicol substitute with an anti-viridal next to its anti-rickettsial spectrum? The microbes which then will be engaged in the production process may well have quite different requirements from those cultivated at present, and new industrial implications will emerge.

To all this, one point should be added. The time has passed when biologists could content themselves with living organisms as they are found in nature. In his scientific fantasia 'Last and First Men; A Story of the Near and Far Future' Stapledon describes how in the age of the 'Third Men' mankind as a whole will satisfy its creative urge in the cultivation of surprisingly new forms of life: on the one hand, creatures of unimaginably perfect harmony; on the other hand, the most

horrifying monstrosities. The biologist of today in his creative powers still ranks far below the votaries of what Stapledon has called the 'vital art', but this does not alter the fact that the microbiologist has already succeeded in producing artificially numerous micro-organisms with quite new property complexes. With the aid of x-rays, ultra-violet radiation, neutrons or of chemical agents it has been possible to bring about radical changes in the wild forms, and many of the mutants thus obtained are characterized by a great stability. Although in most of the cases these mutations will be due to the loss of some genetic unit, the result may be quite beneficial to the industrialist. It is well known that the said procedures have already led to a hundred-fold – or even still higher – increase in yield of penicillin, streptomycin and other metabolic products.

Beadle and his co-workers have further shown that new forms of life may be obtained by applying mutant forms of moulds in hybridization experiments, and Tatum and Lederberg have arrived at the unexpected conclusion that the same should be possible for bacteria.

Finally, it is difficult to overrate the importance of the investigations of Avery and McCarty which brought conclusive proof that the genetical apparatus of a bacterial cell can be reinforced by purely chemical means. Addition of 0.003 microgram of a desoxyribonucleinic acid preparation, isolated from capsulated pneumococcus cells, to the culture of a capsule-less variant sufficed to bring about a permanent restoration of the ability to form capsules in this variant. It is tempting to connect these observations with the virus problem and to conclude that a time may arrive at which, besides virus diseases, virus cures will be known, implying the industrial preparation of various specific desoxyribonucleinic acids.

In any case, it seems certain that the microbiological industry of the future will be a 'key-industry' which will produce and deliver the micro-organisms with optimal properties for technical application.

In concluding I should like to express the hope that the foregoing bird's-eye view of the relations between the microbe world and industry will have convinced you that microbial metabolism has, indeed, multifarious industrial implications.

AN ASPECT OF THE PROMOTION OF SCIENCE

Mr. President, Ladies, and Gentlemen,

I wish to assure you, first of all, that the Royal Netherlands Academy of Sciences feels greatly honored by the invitation addressed to its President to attend the Annual Meeting of the National Academy of Sciences. The Netherlands Academy is proud that, when you decided to make it a tradition to have a foreign academy represented at your meeting – a tradition so admirably inaugurated last year by the President of The Royal Society – your thoughts went out to the Netherlands.

It seems probable that historical considerations were responsible for this. For I need not recall that my countrymen were among the first settlers in this continent, and some of these have either directly, or indirectly by their offspring, left their mark on American history. In a recent speech, Prince Bernhard of the Netherlands cited as an example Klaes Martensen van Roosevelt, a simple farmer of Holland's recently flooded province of Zeeland who arrived in the New World around 1650. Although little is known about this early settler, he became the common ancestor of seven presidents of the United States: James Madison, Martin van Buren, Zachary Taylor, Ulysses S. Grant, William H. Taft, Theodore Roosevelt, and Franklin D. Roosevelt. Altogether there is little doubt that there are nowadays millions of Americans who are at least partly of Dutch descent.

Now that I have used the word 'Dutch', for the first time, I cannot refrain from making a short digression. Only about two months ago, the New York Times thought it fit to devote one of its topics to the use of the word 'Dutch' in the English vocabulary. The article opened with the statement, 'the adjective "Dutch" generally has an opprobrious or uncomplimentary connotation', although, in its usual attempt at fairness, the Times did not omit to observe that, in a few combinations like 'Dutch door', the adjective only denotes the origin of the proto-

type. The Times cited some ten expressions in order to give convincing documentation for its main thesis. My own daily paper commented on the article by remarking that this survey was far from complete: among others, the expression 'to talk double Dutch' was badly missed. I cherish the hope that your abhorrence of this type of speech will make you more tolerant of my imperfect English!

It is far from my thoughts to dwell on this rather baffling use of the word 'Dutch' by way of complaint. In the first place, the incriminating expressions are not of American origin; undoubtedly they are all British-coined and date from the far-off days when the relations between England and Holland were not always of a friendly nature, especially when a Dutch admiral sailed up the river Thames. Secondly, a rebuttal of the article of the Times would be quite out of place, because its tendency was to deplore the misuse of the word 'Dutch' and, above all, because the article wound up with a strong recommendation to send contributions to the treasurer of the Holland Flood Relief at New York. May I seize this opportunity to express to you the profound gratitude of the Netherlands as a whole for the magnificent response American citizens have given to this appeal? In the midst of our deep consternation over the terrible disaster which struck a considerable part of our country, our hearts were warmed by the world-wide sympathy and the very material support given to us by the United States and many other countries as well.

I may not, however, dismiss the discussion of the word 'Dutch' without mentioning here my recent discovery that there are combinations in which the word 'Dutch' apparently has a positively favorable tenor. At least, I came to this conclusion on reading in the kind letter of invitation from your President the revelation of his 'Dutch descent' without any allusion to a possible depreciatory character of this communication.

I hope my audience will forgive me this long digression which may only serve to explain that the pleasant surprise experienced by the Netherlands Academy on receiving your invitation was still enhanced by its contrast to the current evaluation of the word 'Dutch' in colloquial English.

Having been invited to make a speech, I have, of course, meditated on the choice of a suitable subject. It is only natural that I at once felt a strong impulse to avow common ideals, for instance, by enlarging

on the freedom of science or by stressing the social responsibility of the scientist. However, on second thought, I shrank from making topics of such a vital significance for the future of science the subject of a dinner speech. Moreover, in this connexion, I was reminded of the following story:

It is said that one Sunday morning your taciturn President Coolidge, after having attended church, had the following conversation with his wife. Asked whether the sermon had been edifying, Coolidge answered that it had been fine. Not quite satisfied with this general appreciation, Mrs. Coolidge inquired into the purport of the sermon. Coolidge answered that it had dealt with sin. Somewhat exasperated, Mrs. Coolidge asked what the parson had said on the subject. Unhesitatingly Coolidge replied, 'He was against it.'

Now it seems to me that the situation is more or less analogous whenever a subject like the freedom of science is frankly discussed in circles of scientists. Here it will always be possible to summarize the discussion in the words, 'They were for it', although this statement may not do justice to the often very evident differences in the interpretation of what this freedom implies.

I do not wish, however, to be misunderstood. Hence my explicit statement that the Netherlands scientists are fully aware of the grave dangers with which science is confronted nowadays and also that they intently follow your struggle with the clear realization that its outcome may ultimately also decide on their own fate.

Musing on another more suitable theme for my speech, it occurred to me that some reflections on the way of promoting science, this common aim of all academies, might be appropriate. The more so because this might give me the opportunity to include in my speech some remarks on a few Dutch scientists of international fame.

Promoters of science are used to thinking in terms of 'brains and bricks', which slogan, of course, only finds its justification in the pleasant alliteration. Now I think that my audience will agree that providing with 'brains' is a far more difficult problem than that with 'bricks', and I shall, therefore, leave the latter aside. As for the 'brains', it is evident that not every specimen of human brain will do, but that by the term 'brains' is meant the type of brain fertile from the point of view of scientific discovery. Obviously, the primary condition for this type of brain is a well devised scientific education; but, although

its product may be a scientist, there is no certainty that this scientist will belong to those who really extend the frontier of science. This leads to the question, 'What are the characteristics of the truly successful scientist?'

In this connexion, it is tempting to ask your attention for the views of an indisputable scientific genius, the first winner of the Nobel prize for chemistry, the Dutch chemist J. H. van 't Hoff. Three quarters of a century ago, on being nominated to the chair of chemistry at the University of Amsterdam, Van 't Hoff delivered a remarkable inaugural address which bore the title of 'Imagination in Science'. Herein he gave a convincing documentation for his thesis that scientific progress has mainly been due to a strong power of imagination which acts as the driving force in the discoverer. He then concluded that this imagination would as a rule not be restricted to the scientific field but would also manifest itself in artistic talents, love of literature, poetry, romance, etc. He gave a preliminary analysis of the biographies of 200 famous scientists and showed that his hypothesis held true for a high percentage of these men. Of the numerous examples put forward by Van 't Hoff I shall cite only the following: One might expect that James Watt, the inventor of such a practical contraption as the steam engine, would have been a very level-headed man. However, it is reported that, even as a boy, he gave convincing proof of an exceptionally imaginative mind. Once his parents boarded him out for some weeks in a friendly family. When his mother came to fetch him, his hostess said that she was glad to get rid of him. The trouble appeared to be that every night when bedtime was nearing, Watt started to tell pathetic or burlesque stories, one after the other. These tales were so enthralling that he kept the family awake for long hours with the final result that the next day everybody was dead tired.

Van 't Hoff summarized his views on the way in which science progresses in the following quotation from Buckle: 'There is a spiritual, a poetic, and, for aught we know, a spontaneous and uncaused element in the human mind, which ever and anon, suddenly and without warning, gives us a glimpse and a forecast of the future and urges us to seize truth as it were by anticipation.'

In a recent paper by Professor Bernard Cohen of Harvard University, I found the same conviction of the importance of imagination for scientific progress. I need only cite his statement: 'After all, to those

who make the most intelligent scientific guesses we give Nobel prizes.'

What then is this mysterious process of guessing? You may perhaps forgive a microbiologist the fact that he is inclined to draw a parallel with the equally mysterious process of spontaneous mutation, which remarkable phenomenon is nowadays nowhere so notable as in the microbe world. There can be no doubt that in a sufficiently dense clone of certain bacterial strains perpetually forms of life arise which in their properties clearly differ from the single cell which was the starting point of the clone. Among others, these differences may manifest themselves in an unusual resistance to growth inhibitors such as antibiotics, chemotherapeutics, bacteriophage, etc. The main point in all this is, however, that the rise of such a mutant is fully independent of the presence of the inhibitor in the cell's environment, implying that in a clone of sufficient dimensions thousands of mutations are continuously taking place. But all the resulting mutants are doomed to sterility, except the one which quite accidentally fits the environment. This throws a clear light on one of the most remarkable properties of life which might be paraphrased as its 'trial and error' character.

It is tempting to apply this point of view also to the process of guessing. We should then accept that each scientist has a certain intellectual and mental structure and we could consider his guesses as mental mutations. By far the majority of these guesses will remain sterile and vanish, while one guess fitting the scientific environment might stay and mean scientific progress, possibly create a new Nobel laureate. Now it is well known that the mutability of various strains belonging to one and the same bacterial species may vary considerably, and it seems likely that the same will hold good for the mental mutability of the brains of various individuals.

It follows that the task of a promoter of science should be to look out for persons with a marked mental mutability, to try to make them go in for a scientific career, and, above all, to create suitable conditions for their survival as scientists.

Such a program is certainly not easily accomplished, and I shall not consider its initial stages here but limit my attention to the third phase, which involves a selection of those scientists worthy of special consideration from the point of view of scientific progress.

I then start with a recent appraisal of the scientist by A. V. Hill, who writes: 'I do not believe that there is such a thing as the scientific

mind. Most scientists are quite ordinary folk, with ordinary human virtues, weaknesses, and emotions. A few of the most eminent ones are people of superlative general ability, who could have done many things well; a few are freaks, with a freakish capacity and intuition in their special fields but an extreme naiveté in general affairs . . .' Now, it does not seem doubtful that the scientists who are in the first place responsible for the progress of science belong either to the type with 'the superlative ability' or to 'the freakish category'. Nobody will have any difficulty finding characteristic examples of either of the two groups. For me, the outstanding representative of the superlatively able group is H. A. Lorentz, the famous physicist, so well known for his theory of electrons and his contraction theory which initiated the relativistic age in physics. Characteristic of Lorentz was his perfectly harmonious nature which enabled him to deal with equal facility with highly abstract and eminently practical problems. It is not generally known that he devoted himself for almost ten years to the solution of problems connected with the subsequent so successfully accomplished reclamation of the Zuiderzee. But, in addition, he was a master in 'handling' men. As President of our Academy, he has remained unsurpassed, but he scored his main triumphs in international scientific meetings. Accordingly, at the end of his career, he was president of the League of Nations Committee on Intellectual Cooperation, the precursor of Unesco.

You, as Americans, will probably be inclined to think in this connexion of the eminent chemist who now holds the very important post of High Commissioner in West Germany, but many other examples could be given as well.

It will be superfluous to remark that scientists like Lorentz and Conant are not in need of any support from anybody; they are fully qualified to look after themselves!

However, things seem different for Hill's second category of super-normally gifted scientists whom he designates as the freakish type. Now the word 'freakish' has to my ear a somewhat disreputable sound. It is quite possible that I am mistaken in this and that it is only due to my imperfect command of the English idiom. Anyhow I should prefer to call this category, with Ostwald, the romantic type, for any disparagement seems misplaced in view of the huge debt which science owes to scientists of this type.

It is evident that to this category belong the scientists whose imagination and artistic sense markedly surpass the average, the type for which Van 't Hoff in his inaugural address mentioned no less than fifty-two names, all men of well established scientific fame.

Usually these romantic scientists are passionate researchers, often possessed by a true craving for knowledge, which, however, may suddenly subside, the energy of the scientist being directed to other fields of activity. Their mental mutability is apparently highly developed, and, as in the great artists, from time to time instants of genius occur leading to the 'successful guess'. But, as Hill has rightly stressed, they are often marked by an extreme naiveté in general affairs which means that in human society they are exceedingly vulnerable. Here, then, is an obvious task for the scientific promoter: firstly, make conditions such that the persons in question stick to the scientific field; secondly, protect them from adverse conditions emanating from society.

It may be illuminating to pass here in review some passages from the life histories of some successful scientists of the romantic type. This will enable me at the same time to show that, even in the Dutch nation, usually renowned for its soberness of mind, sometimes such scientists arise.

There can be no doubt that Van 't Hoff himself too belongs to the romantic type. In this respect, it is noteworthy that his first publication appeared when, at the age of 22, he worked at the German University of Bonn under Kékulé's direction. It is remarkable that this publication should consist of four pages of English (!) poetry bearing the title 'Elegy on the Death of a Lady Student at Bonn'. The fact that, at that time, an edition of the collected poems of Lord Byron was the favorite book in his library throws some light on this unexpected emotional outburst.

But in the same year, after having returned to Holland, he wrote – and had privately printed – the brilliant pamphlet which made him the founder of stereochemistry. I shall not dwell on Van 't Hoff's further career; it may suffice to state that several circumstances have exerted a stabilizing influence on his devotion to science.

A second example of a typical romantic scientist is my famous predecessor in the chair of microbiology at Delft, M. W. Beijerinck. Those among you who are acquainted with the six volumes of his collected papers will agree that he was one of the truly great microbiologists of

all times. He was the first to bring convincing proof for the bacterial nature of the agent responsible for the formation of the root nodules of leguminous plants. It is well known that this work has had enormous beneficial consequences for agriculture, and the same holds, albeit to a somewhat lesser degree, for his discovery and isolation of the aerobic nitrogen-fixing bacterium *Azotobacter chroococcum*. Symptomatic for Beijerinck's boundless devotion to science is the fact that the latter discovery was made (as appears from his diary) in 1900 on New Year's Eve, *i.e.*, at a time when the thoughts of ordinary people are accustomed to wander in other spheres.

On the other hand, Beijerinck was a perfect example for the naïveté signalized by Hill. Perhaps I can make this at once tangible by quoting from his biography two of his statements. The first runs as follows: 'A man of science does not marry.' The second was an observation on one of his colleagues: 'Mr. So and So gets old, he attends concerts.' It is interesting to trace which factors enabled Beijerinck to survive as a scientist. In 1895, some influential friends and admirers succeeded in inducing the Netherlands Government to erect for Beijerinck a special laboratory at Delft, the first biological institution at any institute of technology in the world. The new chair did not imply any obligatory courses for students, and so Beijerinck managed to lead a protected life for more than a quarter of a century. In a certain period, he was not far from considering his students as mere intruders. It is said that he used to ask them all the same question: 'Are you interested in applied microbiology, such as fermentation industries?' Independently of an affirmative or a negative reply, Beijerinck's retort invariably was: 'Then I have no use for you!' It should be added that the dogged individuals who were not frightened by this reception managed to get in, and, if in addition they gave proof of a serious scientific interest, they were soon introduced into Beijerinck's astoundingly rich spiritual world.

Finally, I should like to dwell for a short moment on the life of one other great microbiologist, *viz.*, on that of Sergius Winogradsky, who died only some months ago at the age of 96. Any one who has read the splendid biography which Dr. Waksman has devoted to the famous Russian investigator will realize that there were many risks that Winogradsky would have been lost for science. Starting as a law student in Kiev, he soon changed to the natural sciences but left these too in order

to study music at the Conservatoire at St. Petersburg. Two years later, he fortunately returned to biology and, under the influence of some eminent teachers, he became deeply interested in the microbe world. Some ten years later, there followed his now classical investigations on autotrophy which added a brand-new chapter to general physiology. This flourishing period in Winogradsky's scientific career was followed by a gradual decline in scientific activity for which troublesome administrative duties may well have been mainly responsible. Being a man of independent means, Winogradsky, in his late forties, decided to retire from the scientific field. He settled permanently on one of his large estates in southern Russia and centered his attention on the practical problems of farm management. He also returned to his old love – the piano – and spent long holidays in Switzerland. Apparently Winogradsky was a total loss for science. Then, in 1918, the Revolution came; Winogradsky fled and, after various wanderings, the sixty-six-year old 'displaced person' arrived in Paris in 1922, where Dr. Roux, the director of the Pasteur Institute, had the happy foresight to offer him the direction of a new division for soil microbiology. The offer was gladly accepted, and this led to the most remarkable result that, in the period 1922–40, a stream of publications appeared which all were written by an aged scientist who for about two decades had evaded the world of science. Nevertheless, these publications revolutionized the science of soil microbiology!

Mr. President, Ladies, and Gentlemen,

I have given you some glimpses of three scientists of the romantic type, and all three stories had a happy ending as judged from the point of view of science. It should, however, be realized that, without a happy ending, there might have been no story at all; or, in other words, it seems quite possible that often potential Van 't Hoffs, Beijerincks, and Winogradskys vanish from the scene in such early stages of their scientific development that their disappearance is scarcely noted.

My first conclusion is, therefore, that we should all be on the lookout for supernormally gifted scientists. If they are of the superlatively able type, we can leave them to themselves. But – and this is my second conclusion – if they are of the romantic type, we should ask ourselves what can be done to stabilize their scientific inclinations and to further their careers.

The first impulse may be to offer them a chair at one of our universities. But here I should like to sound a warning note, which is partly induced by the recent statement on the 'Rights and Responsibilities of Universities and Their Faculties' by the Association of American Universities. It appears to me that this report admirably formulates the creed of the majority of the American scientists as far as the subject of academic freedom is concerned. It is obvious that, also in this report, high demands are made on the mental and ethical structure of the individual faculty members. This point has also been especially considered by Professor Hildebrand in his excellent article on 'The Professor and His Public'. Anyone will agree with his conclusion that 'the public will not accept the claim that academic freedom exempts a man from responsibility to be a decent citizen'. At some other place, Professor Hildebrand explicitly denies the professor the right to be a fool; and again we admit that this is a rightful demand to be made on a university professor, who also has an educational task. But, may we ask the same from a highly gifted scientist of the romantic type? If we remember his 'extreme naiveté in general affairs', I am inclined to answer the question in the negative. This implies that, in view of the indispensability of scientists of the romantic type for the progress of science, very real interests of science will be served, if we can put these scientists at more or less isolated research posts. We should, of course, not give our scientists a license to behave as 'indecent citizens', but, in their infinite worship of science, they should have freedom to ignore citizenship.

It is not the place here to discuss the practical realization of the outlined principle. Suffice it to state that there should be more institutions like your Institute for Advanced Studies at Princeton, more research professorships or fellowships like those sponsored by The Royal Society. Science cannot afford to let scientific geniuses perish, simply because they do not fit the social environment indissolubly connected with the academic teacher.

All the foregoing superficial considerations should, however, not keep me from the main part of my task for to-night. For this can be nothing but to convey a message of friendship, gratitude, and admiration from the Netherlands scientists – and more especially from the members of the Royal Netherlands Academy of Sciences – to the American scientists, of whom my audience is such a brilliant representation.

SOME ASPECTS OF NITRATE REDUCTION

INTRODUCTION

It seems a somewhat risky enterprise to make bacterial nitrate reduction the subject of a contribution to a modern symposium of bacterial metabolism. Most bacteriologists will consider the subject distinctly demoded, and they are fully satisfied with their knowledge of the process. Does not the answer to the question 'nitrates reduced, or not?' yield a diagnostic character in quite common use, and is there not a very convenient routine procedure for the establishment of this characteristic?

Annually thousands of cultures will be grown on broth agar to which 0.1% KNO_3 has been added, and tested for the presence of nitrite in the medium after a few days of cultivation. It is clear that if this test yields a positive result the question 'nitrates reduced' may be answered in the affirmative sense, at least if due precautions have been taken to exclude the possibility that the positive result of the so extremely sensitive nitrite reactions is solely caused by the traces of nitrous acid not seldom present in the laboratory air. Furthermore it is common practice to answer the question regarding the nitrate reducing ability of the organism in the negative, as soon as nitrite can not be detected under the conditions of the experiment. Usually it is not realized that formation of nitrite out of nitrate is only a first step in nitrate reduction, and that there are numerous examples in the literature which testify to the ability of certain micro-organisms to reduce nitrate to further reduction stages, like nitrous oxide and nitrogen. Yet such vigorous nitrate-reducers are not seldom listed as unable to reduce nitrate according to the standard procedure.

The foregoing may only serve as an illustration that the way in which living cells react on the presence of nitrate in their medium may differ considerably, and this suggests at once the desirability of some further analysis of the situation.

In the first place we then encounter the case in which the nitrate taken up from the medium is completely used for the building-up

of cell protein which means that the nitrogen is completely, or almost completely, found back in organic compounds in which the nitrogen occurs in the reduction stage of ammonia. This process can aptly be designated as *assimilatory nitrate reduction*, or simply *nitrate assimilation*.

Next to these there are organisms which bring about a process of nitrate reduction in which the reduction products are mainly excreted into the medium. Here obviously the nitrate is involved in some dissimilatory reaction.

A closer inspection leaves no doubt that in this dissimilatory nitrate reduction two cases can still be distinguished. In the first case the nitrate just interferes with the normal energy yielding process of the cell by acting as many other added hydrogen acceptors would do. This holds for instance, if nitrate is added to various sugar fermenting bacteria like *Bacterium coli*, *Clostridium welchii* a.o. The main effect of the addition is usually a reduction of the nitrate either to nitrite, or to ammonia, which conversion is accompanied by some changes in the nature of the fermentation products. It is clear that this best studied type of nitrate reduction is for the cells in question only of minor importance, and the designation of the process as *incidental dissimilatory nitrate reduction* seems quite adequate.

The full splendour of bacterial nitrate reduction, however, reveals itself in the case in which the nitrate is involved in an energy yielding process which, at least conditionally, is essential for the well-being and proliferation of the organism. It has been definitely established that certain bacterial strains, if inoculated into some well-balanced medium, will die off unless either free oxygen or nitrate is added to the medium. In this case apparently the nitrate can replace the function of oxygen as a hydrogen acceptor in the respiration process, and in doing so the nitrogen of the nitrate consumed is found back in reduced nitrogen compounds.

Here the addition of nitrate decides over life and death of the organism, and it is this *true dissimilatory nitrate reduction* which seems to offer the best chances for a closer investigation, and which for this reason will be exclusively considered in the following.

GENERAL CONSIDERATIONS ON TRUE DISSIMILATORY
NITRATE REDUCTION

The merit of first having clearly described true dissimilatory nitrate reduction goes to the French investigators Gayon and Dupetit [1886], who reported on the results of their extensive and careful studies on nitrogen losses sometimes occurring in water and soil. They established that these losses were due to a conversion of nitrates to molecular nitrogen – or in some cases to nitrous oxide – and demonstrated that this conversion was due to the activity of bacteria which evidently were able to substitute nitrate-oxygen for free oxygen in the oxidation of organic substrates. They introduced the term ‘denitrification’ for those nitrate conversions which lead to the mentioned gaseous nitrogen compounds, but they also found that in certain cases the reduction of the nitrate did not go beyond the nitrite stage, whilst in other cases part of the nitrate-nitrogen was found back in its ultimate reduction stage, *i.e.*, ammonia.

Since then it has been found that the property of true dissimilatory nitrate reduction is not the prerogative of a few very specific bacteria, but is encountered amongst representatives of several genera of aerobic bacteria. Especially in the genus *Pseudomonas* several well-known species are active ‘denitrifiers’. This holds for instance for *Pseudomonas stutzeri* (= *Bacterium denitrificans* β of Gayon and Dupetit), *Pseudomonas aeruginosa* and for certain strains closely related to *Pseudomonas fluorescens* which have been collected in the separate species *Pseudomonas denitrofluorescens*. However, Beijerinck has made part of his classical investigation with an immotile organism not belonging to *Pseudomonas* and which he has named *Micrococcus denitrificans*. In addition true dissimilatory nitrate reductions have been reported for representatives of the genera *Spirillum* and *Bacillus*.

It will be clear that an insight into the true dissimilatory nitrate reduction asks primarily for a thorough study of the fate of the nitrate-nitrogen and of that of the organic substrate acting as the hydrogen donator in the process. As for the former nitrite, nitrous oxide, gaseous nitrogen and ammonia have been found with certainty amongst the reduction products, but these findings are at least partly related to different organisms so that the interpretation of their appearance has remained fragmentary. As for the fate of the organic substrate it is

usually stated that it is completely oxidised to carbon dioxide and water, but the documentation for this statement can scarcely be considered satisfactory. Finally our knowledge of the catalytic systems active in the dissimilatory process is still very incomplete.

FATE OF THE HYDROGEN ACCEPTOR

Almost from the beginning of the study of dissimilatory nitrate reduction it has been clear that the nitrogen of the consumed nitrate can be recovered in different reduction stages depending both on the bacterial species used, and on the experimental conditions. This has naturally led to the idea that these various reduction stages are just intermediates in the conversion of nitrate into the ultimate reduction stage of the nitrogen: ammonia. A critical examination of the experimental evidence for this point of view seems appropriate.

Taking into account that in the dehydrogenation of organic substrate nearly always two hydrogen atoms are removed simultaneously, it is difficult to escape the conclusion that the first step in nitrate reduction will lead to the formation of nitrite:



This view is strongly supported by the fact that in experiments on dissimilatory nitrate reduction as a rule the presence of nitrite in the medium can easily be established, although mostly in small quantities only. It is true that bacterial species have been described which apparently are unable to bring about a further conversion of the nitrite, so that in such a case this compound accumulates in the medium. However, relatively low concentrations of nitrite usually have a toxic effect on the organisms, so that nitrate reduction remains incomplete, thus proving the suicidal character of the process.

Accepting nitrite formation as the first step in nitrate reduction it is only logical to postulate as the second step:



It should be remarked at once that any direct experimental evidence for the correctness of this assumption is lacking, in so far as the occurrence of a compound HNO in a denitrification medium has never been demonstrated. This, however, is not surprising since the studies of Raschig and others leave no doubt that the compound which they

designate as nitroxyl is extremely unstable and liable to various conversions.

So it is quite acceptable that a molecule HNO arising from a reduction of nitrite will be at once subject to a further hydrogenation which will almost inevitably lead to the formation of hydroxylamine:



In the assimilatory nitrate reduction hydroxylamine has frequently been postulated as a precursor of the ammonia or of the amino acid nitrogen in which this element is present in the same reduction stage. I will not enter here into a discussion of the problem whether in these cases hydroxylamine is reduced either before or after the nitrogen has entered into the organic state. It may suffice that the investigations of Virtanen and Csaky [1948] and of Virtanen, Csaky and Rautanen [1949] leave no doubt that in the nitrate assimilation by the yeast *Torulopsis utilis* oxime nitrogen, and also small quantities of free hydroxylamine regularly occur.

For the dissimilatory nitrate reduction by *Denitrobacillus licheniformis* the occurrence of free hydroxylamine has recently been demonstrated by Verhoeven [1952].

The acceptance of hydroxylamine as a precursor of ammonia in those dissimilatory nitrate reduction processes in which this compound is formed seems, therefore, fully justified which means that the last step in the ideal course of nitrate reduction proceeds according to the equation:

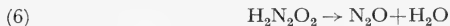


In the preceding discussion the undeniable fact that in dissimilatory nitrate reduction nitrous oxide and nitrogen gas are the main reduction products of the nitrate has been left out of consideration. The most striking aspect of these products certainly is that, in contrast to the nitrate and to the hydrogenation products discussed until now, they contain two nitrogen atoms per molecule. This implies that in some stage of their formation a reaction between two molecules of a hydrogenation product must have occurred.

In this connexion it is clearly indicated to think of nitroxyl for which very unstable compound a spontaneous conversion into a dimer has been well established:



This assumption is the more tempting because the compound $\text{H}_2\text{N}_2\text{O}_2$ could easily be imagined to split off water, hereby yielding nitrous oxide:



Moreover, a continued hydrogenation of the dimer might well yield gaseous nitrogen:



In the meantime the possibility cannot be excluded that the hydrogenation takes place only after the water has been split off, and therefore consists in a hydrogenation of the free nitrous oxide:



The question arises, therefore, in how far experimental evidence in favour of the postulated reactions can be adduced.

It is clear that much would be gained, if we could arrive at an identification of the compound $\text{H}_2\text{N}_2\text{O}_2$. The most plausible hypothesis is that here we are dealing with hyponitrous acid, in itself a far from stable compound, but of which various salts can be prepared without serious difficulties.

Until now, however, all attempts to demonstrate the presence of hyponitrite in denitrification media have failed. Still more important is that it has neither been possible to induce denitrifying bacteria to convert an added hyponitrite preparation either into nitrous oxide or into gaseous nitrogen (Allen and Van Niel, 1952). If, therefore, the preparations in question, indeed, represent the symmetrical configuration which is ascribed to them, hyponitrous acid has to be rejected as an intermediate in dissimilatory nitrate reduction. This situation has led Allen and Van Niel to accept that the compound $\text{H}_2\text{N}_2\text{O}_2$ should have a different configuration, and as such they have postulated nitramide: H_2NNO_2 . This compound differs from hyponitrous acid in the first place by its asymmetrical configuration which – it may be remarked by the way – does not facilitate the explanation of its origination from nitroxyl. In the meantime we owe to Allen and Van Niel the experimental proof that *Pseudomonas stutzeri* is able to bring about a dehydrogenation of nitramide to gaseous nitrogen. This observation certainly adds to the probability that we should specify the reactions (5) and (7) as follows:



In recent studies there is an increasing tendency to detract from the importance of nitrous oxide as a regular product in dissimilatory nitrate reduction. Sacks and Barker [1952] state explicitly that they were unable to detect any nitrous oxide in their denitrification experiments with their strain of *Pseudomonas denitrificans*. Moreover, they conclude that nitrous oxide cannot be a normal intermediate in the formation of nitrogen, because its utilization may be selectively blocked by azide or dinitrophenol under conditions which permit the formation of nitrogen from nitrite, and because a lag frequently precedes the utilization of nitrous oxide by resting cells, which lag is not found in the conversion of nitrite into nitrogen. In their extensive study of dissimilatory nitrate reduction by *Pseudomonas stutzeri* Allen and Van Niel are initially inclined to hold the opposite view, since they established a simultaneous enzymatic adaptation to nitrate, nitrite and nitrous oxide. However, they finally reject the idea on basis of the observation that the conversion of nitrous oxide into nitrogen is inhibited by 3×10^{-4} m cyanide, whilst under the same conditions nitrite is not converted into nitrous oxide – as might be expected on the basis of the intermediate theory – but quite normally into nitrogen.

The arguments of both groups of American investigators seem convincing. There may, perhaps, be one way of escape. Apparently the possibility has not been considered that in the nitrite containing media azide and cyanide in the very low concentrations in which they are used are either completely or at least partly removed by a chemical reaction with the excess of nitrite. If this would apply, nitrous oxide could be restored in its position of a regular precursor of gaseous nitrogen.

For the moment we may accept that at least under certain conditions nitrous oxide does not precede nitrogen, implying that nitrogen gas can originate from a direct hydrogenation of the compound $\text{H}_2\text{N}_2\text{O}_2$ according to equation (7).

On the other hand I wish to emphasize that in certain cases there is not the slightest doubt that nitrous oxide is a direct precursor of nitrogen in dissimilatory nitrate reduction. The experimental proof for this statement has been brought for bacterial species which under normal cultural conditions are more disposed to produce nitrous oxide than holds for both *Ps. denitrificans* and *Ps. stutzeri*. A special

quest in this direction has shown that *Pseudomonas aeruginosa*, *Micrococcus denitrificans* and *Denitrobacillus licheniformis* all produce nitrous oxide under normal conditions in fairly large quantities.

For the last-mentioned species Verhoeven [1952] has definitely shown that in growing cultures in the first phase of the process up to 65% of the nitrate is converted into nitrous oxide, whilst in the second phase the greater part of this compound disappears and is converted into nitrogen. It should, however, be emphasized that at the stage of the maximum yield of nitrous oxide only a small part of this product was present in the gas phase, about four fifth of it has remained dissolved in the medium, from which it was almost spontaneously set free in the vacuum applied in the Van Slyke gas chamber. In various respects this is a remarkable observation. In the first place it means a solution for a riddle already signalized by Korsakova [1927], and amply confirmed in several of Verhoeven's experiments, that quite considerable nitrogen losses occur in certain stages of denitrification processes when the media are analysed with the aid of the usual methods, taking into account the gas evolved. In making such nitrogen balances until now no attention had ever been given to the nitrous oxide dissolved in the medium.

But the observation is particularly remarkable when considered from a quantitative point of view. For it was found that the amount of nitrous oxide which could be extracted from the medium surpassed at least several times the solubility of this gas in pure water as determined by Schwab and Berninger [1928]. We must, therefore, conclude that nitrous oxide originating in an aqueous medium has a strong tendency to form supersaturated solutions, unless we are willing to accept that in the medium nitrous oxide is present in some unstable compound (hyponitrous acid?) which at once dissociates with nitrous oxide evolution, as soon as the partial pressure of the nitrous oxide in the gas phase is markedly reduced. Whatever the explanation may be, this does not do away with the irrefutable fact that in the second phase of a normal denitrification experiment streams of nitrogen escape from a medium which contains neither nitrate, nor nitrite. There can, therefore, be no doubt that under certain conditions either free nitrous oxide itself or some unstable compound which easily dissociates under nitrous oxide formation is the source from which gaseous nitrogen arises.

A further demonstration of this statement was obtained in an exper-

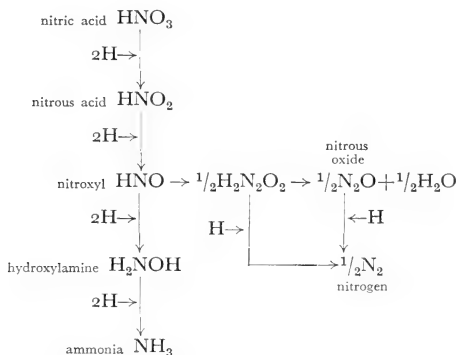
iment in which the gas production was determined in three identical cultures of *Denitrobacillus licheniformis* in which the partial pressure of nitrous oxide in the gas phase was varied. In one culture the gas could escape at normal atmospheric pressure, in the second one the partial pressure was reduced at regular intervals by evacuation, whilst in the third case the culture was brought in a thick-walled sealed tube, under such conditions that the final pressure of the gas surpassed 10 atmospheres. The influence of the various measures on the composition of the gas evolved is shown in Table I; the figures given leave

TABLE I

The influence of the partial pressure of nitrous oxide on the composition of the gas evolved in a denitrification experiment with *Denitrobacillus licheniformis*

	Gas evolved in ml		Composition gas in %	
	N ₂	N ₂ O	N ₂	N ₂ O
Vacuum	16	32	33,3	66,7
1 Atmosphere	42	8	84,0	16,0
Pressure	52	1	98,2	1,8

no doubt that with increasing partial pressure of the nitrous oxide in the gas atmosphere this gas is to a larger extent converted into nitrogen.



It is worthwhile to add that this behaviour is not at all restricted to *Denitrobacillus licheniformis*. On studying the denitrification process caused by *Pseudomonas aeruginosa* with glucose as hydrogen donor it was found that under normal conditions the nitrous oxide content of the gas evolved always remained below 20%. However, on applying the vacuum technique nitrous oxide contents of 70% and higher were regularly observed. It may be remarked that this evidently means a new and elegant way of trapping an intermediate product in metabolism.

All the foregoing considerations taken together seem to justify the reaction scheme (preceding page) as given by Verhoeven [1952].

FATE OF THE HYDROGEN DONATOR

The unmistakable analogy between the bacteriological processes of sulphate, of carbonate and of nitrate reduction seemed to suggest that also in the latter process incomplete dehydrogenations might occur. For sulphate reduction this phenomenon had been discovered by Baars, and Barker found an analogous situation in carbonate reduction, the process better known as methane fermentation. On the other hand nitrate reduction differs from the two other processes by the fact that the occurrence of incomplete hydrogenation stages of the nitrate is quite common, whilst in sulphate reduction only the ultimate hydrogenation product of sulphur: SH_2 , and in carbonate reduction only the analogous hydrogenation product of carbon: CH_4 are encountered. This situation suggests the possibility that in this type of processes incomplete dehydrogenation and incomplete hydrogenation are mutually exclusive.

As far as known the only publication in which the fate of the organic hydrogen donor in true dissimilatory nitrate reduction has been the subject of a thorough investigation is the article by Sacks and Barker [1952]. These authors made carbon balances of the denitrification caused by *Pseudomonas denitrificans* in a medium in which sodium succinate and glutamic acid were the only organic compounds present. The results were quite convincing: approximately 90% of the carbon of the organic substrates consumed was found back as carbon dioxide; the remaining 10% could be accounted for as the carbon present in the bacteria formed.

Independently this problem had also been attacked in my laboratory by Mr. J. J. C. Goos. Since in these experiments a different bacterial species, *viz.*, *Pseudomonas aeruginosa*, and also a different hydrogen donor, *viz.*, glucose, have been used, it seems worth while to report the results obtained, the more so because also oxido-reduction balances have been drawn up.

As for the carbon balances the results have been collected in Table II:

TABLE II

Carbon balance in nitrate reduction by *Pseudomonas aeruginosa* with glucose as hydrogen donor

Glucose consumed	Recovery as CO ₂	Recovery in bacteria*	Total recovery	Recovery in % of carbon in glucose consumed
(mg)	(mg)	(mg)	(mg)	
106	76	35	109	103
101	72	32	104	103
97	69	26	95	98
92	72	27	99	107
195	142	60	202	104

* Calculated from protein content, assuming both protein and carbon content to be 50% of dry weight.

It should be remarked that the amount of carbon present in the bacteria has been estimated by an indirect method. At the end of the experiments the amount of protein present has been determined by the Kjeldahl-method. Since both the protein content and the carbon content of the dry bacteria may be assumed to be about 50%, this means that in first approximation the two values are interchangeable. The errors introduced by this procedure can only be of minor importance, and the figures leave no doubt that the carbon of the glucose consumed, in as far as not recovered in the bacteria, is almost quantitatively found back as carbon dioxide.

The oxido-reduction balances support this conclusion.

It seemed, nevertheless, dangerous to generalize this conclusion, because we know of several instances in which of two related bacterial

species one brings about a complete, and the second one an incomplete dehydrogenation of an organic substrate (acetic acid bacteria, sulphate reducing bacteria, etc.). For this reason we have extended these experiments to both other bacterial species, and other substrates.

In this respect Verhoeven has given special attention to various alcohols. It should be remembered that Baars in sulphate reduction, and Barker in carbonate reduction established an almost quantitative conversion of ethanol into acetic acid. Using *Pseudomonas aeruginosa* Verhoeven met with partial success, in so far as he succeeded in detecting acetic acid, propionic acid and acetone as incomplete dehydrogenation products in media containing ethanol, propanol and isopropanol respectively. It should be added, however, that the amount of the said products corresponded only with a small fraction (2.5% maximal) of the substrates consumed. Nevertheless, these results bring a first indication that also in dissimilatory nitrate reduction the dehydrogenation occurs step-wise. Of course, there still remains the unsolved problem why in sulphate and carbonate reduction the incomplete dehydrogenations, and in nitrate reduction the incomplete hydrogenations prevail.

MOLECULAR HYDROGEN AS HYDROGEN DONATOR

There is one hydrogen donator which asks for a special consideration, viz., molecular hydrogen. That this gas can be utilized in incidental dissimilatory nitrate reduction has already been shown several years ago by Stephenson and Stickland [1931] and by Woods [1938]. The latter author proved definitely that resting cells of *Clostridium welchii* were able to reduce nitrate to the ultimate reduction stage: ammonia. This result does, however, not at all imply that hydrogen can also be a suitable donator in true dissimilatory nitrate reduction, for it may be deemed certain that it is impossible to devise a medium which does not sustain the anaerobic growth of the said bacterial species, but which is able to do so on addition of nitrate.

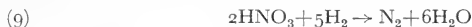
The same holds for the hydrogen oxidizing bacterium recently described by Schatz and Bovell [1952] under the name *Hydrogenomonas facilis*. Although resting cells of this bacterium in a hydrogen atmosphere bring about a vigorous reduction of nitrate to the nitrite stage, it is described as a strictly aerobic organism. Here again thus we are

dealing with a typical case of incidental dissimilatory nitrate reduction.

For our purpose it is only of importance to consider the question whether bacteria which can derive their energy from the system organic hydrogen donator and nitrate can do so also from the system molecular hydrogen and nitrate. The first indication that such organisms exist can be derived from an observation of Lebedeff [1909] who established that in cultures of bacteria oxidizing molecular hydrogen with free oxygen the nitrate present in the medium was also reduced and partly converted into gaseous nitrogen. The subject was more systematically studied by Niklewski [1914] who in several publications gave a convincing demonstration that a pure culture of his *Hydrogenomonas agilis* is able to derive its energy from the system: hydrogen/nitrate. Niklewski emphasizes that his bacterium is also characterized by a heterotrophic mode of life as far as free oxygen is available. Although Niklewski is inclined to conclude that *H. agilis* can reduce nitrate with molecular hydrogen only, the documentation for this statement is very meagre.

A critical examination of this point did not seem to be superfluous, and as a first step to this it seemed worth while to investigate whether typical denitrifying bacteria which are able to use very different organic hydrogen donators can also use molecular hydrogen in nitrate reduction.

This investigation was made by Mr. A. L. Koster. For a first quick orientation the 'resting cell' technique seemed most suitable; one might expect that denitrifying organisms which could derive their energy from the molecular hydrogen/nitrate system would in any case be able to bring about in the Warburg apparatus the conversion:



However, from earlier manometric studies on denitrification (Van Olden, 1940), later confirmed by Pollock [1946] and by Wainwright and Pollock [1949] it has resulted clearly that the nitrate reducing enzyme: nitratase is a typical adaptive enzyme, so that resting cells only reduce nitrate without considerable lag, if they have originated in a nitrate containing medium. Moreover, an analogous situation has been proved to exist in the bacteria oxidizing hydrogen with free oxygen for the enzyme system which activates molecular hydrogen: hydrogenase.

For these reasons the various denitrifying bacteria tested in the 'resting cell' experiments were all grown in a hydrogen atmosphere on an agar medium containing 1% peptone and 2% potassium nitrate.

The results of these experiments, in which *Pseudomonas aeruginosa*, *Ps. denitrofluorescens*, *Ps. stutzeri*, *Micrococcus denitrificans* and *Bacterium vulpinum* were tested, were, however, entirely negative. Pressure changes were practically the same whether or not nitrate had been added to the bacterial suspension, and whether the atmosphere consisted of hydrogen or of nitrogen.

The negative outcome of these experiments strongly suggested that, if bacteria exist which can bring about a denitrification with molecular hydrogen, they are of a specific type, and do not belong to the group of better known denitrifiers. This has induced Mr. Koster to start enrichment cultures in a medium containing nitrate and other essential mineral salts under conditions in which molecular hydrogen was the sole hydrogen donor available. This implied the absence of all organic compounds so that in other words only autotrophic bacteria could develop.

Some garden soil was inoculated into a somewhat modified Eldredge tube containing a mineral medium as prescribed by Grohmann [1924] for the cultivation of his 'Knallgasbakterien' with the addition of 2% KNO_3 . The air in the vessel was then removed by a mixture of 15% CO_2 and 85% H_2 . The culture vessel was continuously shaken at a temperature of 30 °C.

After two to three days a considerable reduction of gas pressure could be established. However, attempts to isolate a hydrogen absorbing bacterium by streaking on an agar medium containing the mineral constituents used by Grohmann with addition of 2% KNO_3 – replacing the distilled water by tap water – and cultivating in the CO_2/H_2 mixture were unsuccessful. No growth occurred. Moreover, transfers directly from the enrichment culture into fresh liquid media of the same composition also quickly lost their hydrogen absorbing capacity.

The successful start and the so disappointing continuation of the experiment seemed to indicate that the hydrogen consuming organism in the enrichment experiment was not a strict autotroph. This consideration led to the attempt to isolate the organism on the medium earlier applied, however, with addition of 0.3% yeast autolysate. This

time good growth of a uniform type of colonies occurred, and by replating a pure culture was obtained which proved to be quite active in the original liquid medium, provided that here too some yeast autolysate was added. For this pure culture it was found in Warburg experiments in which a limited amount of nitrate was added that the reduction in gas volume was in excellent agreement with what should be expected on the assumption of a reduction of nitrate to gaseous nitrogen according to equation (9). Herewith for the first time experimental proof has been brought that there exist bacteria which can use molecular hydrogen as a hydrogen donator in nitrate reduction leading to nitrogen.

A further study of the pure culture in question showed at once that the organism was not at all a specific autotrophic form of life. A vigorous denitrification could also be obtained with a large number of the more commonly used organic hydrogen donators. Moreover, the bacterium also showed excellent development under aerobic conditions on peptone media with and without glucose, and in the absence of nitrate.

Microscopic examination showed immotile coccus- to spindle-shaped, Gram-, non-sporeforming cells. This together with its physiological properties made it tempting to identify the organism with the bacterium described by Beijerinck as *Micrococcus denitrificans*. It is true that there remained one difference, *viz.*, that the type strain of the species had been found unable to reduce nitrate with molecular hydrogen.

However, the studies on 'Knallgasbakterien' [Ruhland, 1924; Grohmann, 1924; Kluyster and Manten, 1942] have shown that the property of activating gaseous hydrogen is in these bacteria easily – and often apparently irreversibly – lost. Under these conditions there seems to be no reason to reject the proposed identification.

Although Schatz and Bovell [1952] report that in their *Hydrogenomonas facilis* hydrogenase is present independent of cultural conditions, it seemed worth while to study for the newly isolated strain in how far the ability to reduce nitrate with molecular hydrogen was dependent on the preceding cultural conditions.

In a series of experiments with resting cells performed by Mr. M. C. A. van Nieuvelt it became evident that for a realization of nitrate reduction with molecular hydrogen at least three conditions have to be

fulfilled as regards the previous history of the cells. Only those cells proved to be active if originated from cultures grown:

1. on media containing nitrate;
2. under anaerobic conditions in a hydrogen atmosphere;
3. on media poor in organic compounds.

The first condition is a confirmation of Van Olden's observations on the adaptive character of the nitratase.

The second condition proves that the same holds for the hydrogenase, the enzyme system activating molecular hydrogen, a result which conforms with the analogous behaviour of 'Knallgasbakterien' as described by Kluver and Manton [1942].

The third condition is the most surprising, but Fig. 1 leaves no doubt that there exists a strong contrast, as far as hydrogen absorption is concerned, between cells cultivated on Grohmann's medium with nitrate and those grown on peptone agar + 1% KNO_3 both in a hydrogen atmosphere.

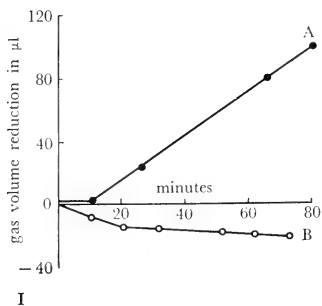
We must, therefore, conclude that production of hydrogenase is suppressed, if the bacterium can rely on organic compounds as sources of active hydrogen. The exactingness of the bacterium as regards hydrogenase production contrasts strongly with the behaviour of Schatz and Bovell's *H. facilis*.

The results obtained opened the possibility that the negative outcome of the hydrogen consumption in the Warburg experiment with the authentic strain of *Micrococcus denitrificans* might have been due to the fact that the bacteria had been cultivated on a medium containing peptone.

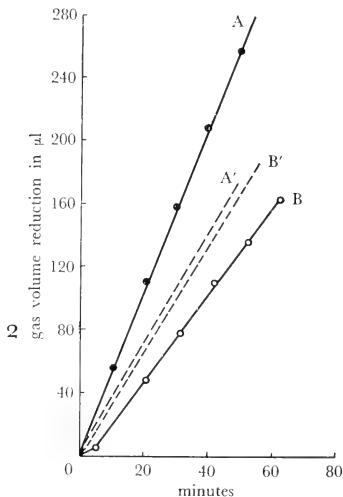
In accordance with this assumption it was, indeed, found that the strain in question was able to bring about a vigorous hydrogen absorption, if inoculated into a Grohmann medium with 2% KNO_3 and 0.3% yeast autolysate.

Herewith the last obstacle against an identification of Koster's bacterium with Beijerinck's *Micrococcus denitrificans* has been removed.

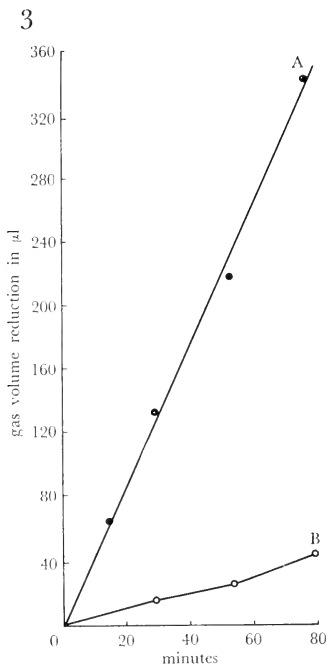
The ability to utilize molecular hydrogen as a hydrogen donor in nitrate reduction suggested an inquiry into the possibility that the bacterium could also use hydrogen gas in aerobic metabolism, *i.e.*, with free oxygen as hydrogen acceptor. This proved, indeed, to be the case, as is shown in Fig. 2, in which curve A gives the gas absorption



I



2



3

Fig. 1. Hydrogen consumption with nitrate as acceptor. Curve A, bacteria grown on Grohmann medium with nitrate. Curve B, bacteria grown on peptone agar with nitrate both in a hydrogen atmosphere.

Fig. 2. Hydrogen consumption with either O_2 or nitrate as acceptor by bacteria grown on Grohmann medium with nitrate in a hydrogen atmosphere. Curve A, gas change in 50% H_2 , 10% O_2 and 40% N_2 in the absence of nitrate. Curve B, gas change in hydrogen with nitrate. Curves A' and B', give the net hydrogen consumption in the two cases.

Fig. 3. Hydrogen consumption with either O_2 or nitrate as acceptor by bacteria grown on Grohmann medium without nitrate in an atmosphere containing H_2 and O_2 . Curve A, gas change in 50% H_2 , 10% O_2 and 40% N_2 in the absence of nitrate. Curve B, gas change in hydrogen with nitrate.

by cells – grown in hydrogen on a Grohmann medium containing nitrate – in an atmosphere containing 50% H_2 , 10% O_2 , 40% N_2 in the absence of nitrate, and curve B the gas absorption by the same cells in a medium containing KNO_3 in an atmosphere of pure hydrogen. Taking into account that in the first case the amount of hydrogen consumed is only two thirds of the gas consumption observed, and in the second case five fourth of this consumption, it is clear that in both conversions the hydrogen consumption is practically the same, implying that the capacity of the hydrogenase is in both cases controlling the rate of the reaction.

Fig. 3 dealing with cells grown on a Grohmann medium without nitrate in an atmosphere containing both H_2 and O_2 shows that under these conditions the cells maintain the ability to catalyze the 'Knallgasbakterien', and therefore contain hydrogenase (curve A), but lack of nitratase practically prevents nitrate reduction with hydrogen (curve B).

We may summarize the foregoing by stating that the heterotrophic denitrifying bacterium *Micrococcus denitrificans* produces under certain conditions a hydrogenase which enables it to use molecular hydrogen as a hydrogen donator in nitrate reduction. However, both nitratase and hydrogenase are typical adaptive enzymes, and therefore resting cells are only active, if both nitrate and hydrogen were available in their cultivation. The bacterium can at the same time transfer hydrogen to free oxygen, or in other words it belongs to the physiological group of 'Knallgasbakterien'. To a certain extent this heterotrophic bacterium can be considered as a facultative autotroph, although evidently certain organic growth substances are essential.

THE INFLUENCE OF FREE OXYGEN ON NITRATE REDUCTION

For all bacteria capable of a true dissimilatory nitrate reduction it is characteristic that this property is a facultative one, in as far as they are able to proliferate as well in the absence of nitrate, provided that free oxygen is available. In other words nitrate and free oxygen are interchangeable as hydrogen acceptors. This point of view – which contrasts strongly with what holds for sulphate and carbonate reduction – lends a special interest to a study of the influence of free oxygen on dissimilatory nitrate reduction.

In most textbooks it is stated that even low concentrations of oxygen are able to suppress dissimilatory nitrate reduction, a conclusion to which much importance is attached, since it tends to imply that notwithstanding the ubiquity of denitrifying bacteria in various soils nitrogen losses from nitrate do not occur, if a sufficient aeration of the soil is warranted.

In later years several authors, Korochkina [1936], Meiklejohn [1940], Korsakova [1941], Sacks and Barker [1949], Skerman *et al.* [1951] and Verhoeven [1952] have convincingly shown for quite divergent types of denitrifying bacteria that only a very thorough aeration of the cultures is capable of suppressing denitrification. With the aid of the polarographic method Skerman *et al.* have clearly demonstrated that in growing cultures nitrate reduction does not commence before the oxygen concentration in the medium is reduced to 'zero', and in addition they have shown that only an energetic stirring of the culture in contact with air suffices to maintain a measurable concentration of oxygen in the medium.

We must conclude from their experiments that even very small amounts of oxygen, indeed, at once suppress denitrification. Whether this is due to a direct inactivation of the nitrate reducing enzyme by the oxygen, or whether the respiration process as such in some way interferes with the nitrate reduction must be left undecided.

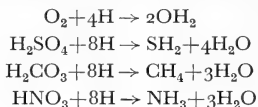
Although the extreme sensitivity of nitrate reduction towards free oxygen is in itself very reassuring from the point of view of nitrogen losses in arable soils, on the other hand the demonstration of the difficulty of maintaining oxygen tensions at places of active bacterial metabolism leaves no doubt that not seldom in micro-elements of soils oxygen tension will be zero, thus opening the possibility of denitrification.

A most remarkable result of Verhoeven's investigation was that oxygen had a most unexpected effect on the metabolism of various strains of *Denitrobacillus licheniformis*. In studying the disappearance of nitrate in shallow layer cultures with surface aeration, it was found that a surprisingly high percentage (sometimes 90%) of the nitrogen of the nitrate consumed was under these conditions converted into its ultimate reduction stage: ammonia. Also in this case only the vigorous aeration obtained in shake cultures led to a preservation of the nitrate.

Taking into account that under anaerobic conditions *Denitrobacillus*

licheniformis converts nitrate into a mixture of nitrogen and nitrous oxide, the fact that under moderate aeration these reduction products are replaced by ammonia favours the idea of a common precursor for both types of products, as accepted in the scheme given above. In some way free oxygen should then counteract the dimerization of the intermediately formed HNO , thus preserving it for further hydrogenation.

How this may be, Verhoeven's observations on the ammonia production from nitrate brings the dissimilatory nitrate reduction fully in line with dissimilatory processes like respiration, sulphate and carbonate reduction, as is evidenced by the following equations:



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MICROBE AND LIFE

THE privilege to address the combined sections at the annual plenary session poses for the speaker a difficult problem in connexion with the choice of his subject. The result of my assiduous meditations has been that I shall attempt to give you an inkling of the somewhat special attitude concerning the phenomenon of life that unavoidably develops in those who for many years have been preoccupied with a study of the microscopically small organisms. Hence I might justifiably have announced my discourse as 'the microbiologist's philosophy of life', if it were not for the fact that this might have entailed the danger of creating on the one hand a misconception, and on the other an anticipation that would not be fulfilled. This may be explained in the following manner.

The word 'microbe' is a neologism; not until 1878 was it introduced into scientific vocabulary by the surgeon Sédillot, who had first sought and obtained the high sanction of the great philologist Littré. In spite of this the term microbiologist, designating the student of the *logos* of the *microbia*, is still not infrequently interpreted as indicating a biologist of small stature. So much for the misconception; as for the anticipation, the phrase 'philosophy of life' has gradually acquired a restriction – and at the same time a greater profundity – that causes its meaning to deviate markedly from a simple contemplation of life, considered as the aggregate of the enormously divergent manifestations of life. Consequently I have chosen a less ambitious title, just sufficient to serve as a warning to prospective auditors. What I wish to discuss may perhaps be adequately summarized as 'the microbiologist's concept of life'.

The first item that contributes greatly to the microbiologist's concept of life is the ever vivid recognition of life's ubiquitousness. The fact that various living germs lie dormant on so serene an object as our chairman's gavel, for example, immediately throws a startling light on the potencies of life in the aggregate, and this the more so because it is coupled with the experience that the word 'dormant' must in this

connexion be considered as an euphemism; in many respects it would be better to indicate the situation by saying that the germs are lying in wait. For often it are merely slight changes in environmental conditions that suffice to rouse this dormant life to abundant activity which leads to a rapid multiplication of the living units. Usually this process is associated with changes in the life-inducing substrate so profound that these cannot escape being observed even by the layman. I need but remind you of the cases of food spoilage known to all of you from domestic experience. And that the substance of the chairman's gavel is no less exposed to potential menace is something of which every one can immediately be convinced by paying a visit to the lofts of the 'Trippenhuys' where it will be noted how drastically the solid wooden rafters of Justus Vingboons creation have fallen prey to the activities of microbial life.*

Nevertheless, as documentation for the ubiquitousness of life the incidentally chosen example of a physically rather unimpressive object such as the chairman's gavel may perhaps inspire you but little. Let me therefore state that the microbiologist has encountered living organisms in atmospheric strata some miles high, as well as in oceanic sediments more than six miles below sea level; furthermore, that he has found them in the water of the Dead Sea which, on account of the high concentration of various salts, has acquired a bad reputation, although the predicate 'dead' is evidently not applicable; and likewise in hot springs so warm that they would scald the human hand.

A second aspect of life with which the microbiologist is conversant is the surprisingly great quantitative significance of the microbial world. It is known that our green plant kingdom on which man and animals depend for their existence draws its nourishment from the air, absorbing atmospheric carbon dioxide, and liberating oxygen. Reliable estimates show that the intensity of this carbon dioxide assimilation by the existing flora is so great that the carbon dioxide content of the atmosphere would be depleted in some 30-40 years unless carbon dioxide would be returned to the air in some manner or other. Now it is quite certain that this supplementation is largely due to the fact that in nature the dead plant material is reconverted into carbon

* The 'Trippenhuys' is the name of the building occupied by the 'Koninklijke Nederlandse Akademie van Wetenschappen'; it was built in 1660-1664 by Justus Vingboons as a domicile for the Trip family.

dioxide. Already at an early date the respiratory process of man and animals has been marked out as such a source of carbon dioxide. A century ago Pasteur emphasized that the conversion into carbon dioxide of that part of dead vegetable matter that is not used as animal food proceeds under the influence of the microbial world. Tersely he formulated this idea in the statement: 'C'est encore la vie qui préside au travail de la mort'. Our late fellow-member, Eugène Dubois, who so often spoke in this very hall, was probably the first to venture an estimate of the extent to which animals and microbes, respectively, participate in this carbon dioxide production. Dubois concluded that the former is of an order of magnitude of only 2 per cent. And because during the historical era an equilibrium between production and utilization has evidently existed, this implies that the remaining 98 per cent must largely be ascribed to microbial activity. Even though these figures may be in need of some correction, this situation entails the inevitable consequence that, as far as vital processes are concerned, the bulk of matter of microbial origin must exceed that of the animal kingdom by many times. Thus the microbiologist is imbued with the notion that perhaps more than one-half of life, *i.e.*, of the totality of vital phenomena, escapes the attention of the unsuspecting observer. This might also be paraphrased as follows: the major part of life on earth has, so to speak, 'gone underground', implying that to the microbiologist has been assigned the task of studying the macromoiety of life.

Another characteristic feature of life that forces itself particularly on the microbiologist is the indissoluble interrelatedness of all living creatures which is, for example, manifest in the indispensability of the microbial world for plants and animals. If someone were to possess a magic force with which at one fell swoop he could destroy exclusively the single-celled forms of life, there would be hardly any immediately apparent changes. But very soon disastrous consequences would emerge. All those areas where plant life flourishes, all the woods, meadows, etc., would be changed into arid regions in the course of a few years. On an earth where *Homo sapiens* had not yet made his appearance this would inevitably imply an early cessation of animal life as well. Only the circumstance that in a later phase of terrestrial evolution the human intellect must also be taken into account forces me to make some reservations concerning the present.

After Reyniers has demonstrated the possibility of a microbe-free existence for monkeys, we may assume that this would equally apply to man. In principle he might thus manage, by combustion of the dead vegetable matter, and with the aid of his fertilizer industry, to keep up at least part of the vegetation, and hence also part of the animal world. But only a burdensome existence would allow him to maintain this unnatural community in a restricted area; and the lack of foodstuffs such as butter, bread, and cheese, and of luxuries such as beer, wine, and liqueurs, would further enhance the drabness of this existence. From these considerations the microbiologist acquires an appreciation of the remarkable interrelatedness of all forms of life, utterly divergent as they are; and in due course he is forced to the notion that there must be a fundamental unity at the bottom of it.

However, the explorations which the exponents of experimental microbiology have undertaken in the microbial world during the century of the existence of this science have initially led to the emergence of quite different points of view. For there soon appeared to be a bewildering diversity of living organisms with which in the early days the investigator could familiarize himself only with the aid of his microscope, although gradually he also became acquainted with their stupendous activities after he had learned to cultivate these organisms in his laboratory. Yet it was not so much the wealth of shapes that caused the astonishment; rather was it the ecological observations that confused his mind. In particular did this apply to the experience that microscopic life can flourish under conditions under which neither the green plants nor the animals can live. It is no exaggeration to say that the pertinent and extensive studies have added an entirely new chapter to general physiology. The following remarks may serve as an, admittedly superficial, documentation of this statement.

From early times the biologist had experienced that every system that may lay claim to the predicate 'living', with the exception of some special cases of dormant stages, is characterized by its dynamic nature which manifests itself for instance in the phenomenon that is generally referred to as metabolism. This is predicated on the empirical fact that for its continued existence and eventual multiplication every living cell depends upon a continuous supply of certain foodstuffs that undergo chemical transformations which yield partly cellular building blocks, and partly products that the cell excretes into

its surroundings. It are these latter conversions that constitute the basis for the energy-requiring functions of the living organism.

In surveying the situation in green plants and higher animals from this point of view the following main features can be delineated. The green plant derives its energy primarily from solar radiation which permits it to synthesize its cell material from the inorganic carbon dioxide and a few other mineral ingredients. In contrast, life in the animal kingdom has exploited an entirely different source of energy; the chemically so extremely varied plant material provides the animal's nutrition, and part of the food components furnish the requisite energy as a result of the slow combustion during the so-called respiratory process. These oxidizable substrates tend to belong primarily to the groups of carbohydrates, fats, and proteins, quite familiar to us from human dietetics.

Now the scientist who, armed with this knowledge of the basis on which rests the life of plants and animals, investigates the corresponding situation in the world of the microbes, is apt to experience one shock after another. It is true that occasionally he will encounter some groups of micro-organisms whose metabolic features correspond essentially to those of the green plants and animals, respectively, but for the vast majority of microbial types that also multiply profusely in the absence of light the conditions for animal metabolism appear not to be realized in the least. A first surprise comes with the finding that for many bacteria the organic foodstuff, and hence also the substrate for respiration, may be limited to one single organic substance, and that this may even be a member of some class far removed from the groups of carbohydrates, fats, and proteins.

Far more surprising is, however, the fact that frequently any one of a vast number of compounds, quite divergent in chemical respect, can serve as the sole organic food constituent for one and the same bacterial species. If one ponders the nature of such nutrient substrates, the astonishment ever increases. For what should one think of bacteria that apparently feel quite comfortable if they are provided with carbon monoxide as the exclusive respiratory substrate, *i.e.*, with the substance, designated as 'coal gas', that has acquired such a bad reputation in human society because its presence in very low concentration in the air soon terminates all human and animal life? Still more astounding is perhaps the demonstration that among the accept-

able substrates for life one also encounters chemical compounds that do not naturally occur and owe their origin entirely to the chemist's ingenuity.

And yet, this is hardly more than a first step in the direction of a sketch of the marvellous capacities of microbial life. How reverently must we bow our heads before the potentialities of cells that manage to synthesize their various and numerous cell constituents from the simple carbon dioxide molecule exclusive of a radiant energy supply, and satisfy their energy requirements at the expense of some strictly inorganic chemical system. Such systems may be composed of mixtures of oxygen with, as oxidizable component, for example, sulphur, iron, ammonia, or nitrite. A record achievement in this respect is perhaps furnished by the so-called hydrogen bacteria where we observe life being sustained by the simple conversion of hydrogen and oxygen to water.

Then we must also consider the following facts. The view had long been held that, apart from the green plants which themselves produce oxygen with the aid of solar energy, the presence of free oxygen is a prerequisite for the maintenance of life. Absence of this gas usually causes an early death of the animal organism. Once again it has been the exploration of the microbial world that has liberated life from this limitation. Pasteur discovered the phenomenon of what he pithily termed '*la vie sans air*', with the mighty implication that life is not restricted to the atmosphere or to environments that are in direct contact with it, such as the hydrosphere, but can penetrate into the deeper layers of the lithosphere.

Compared with the above-mentioned, hardly comprehensible diversity in physiological types found among micro-organisms, the corresponding mutual differences encountered among the higher plants and animals become negligible. Hence the microbiologist is always aware of the existence of impressive potencies of life that are all too frequently ignored. In consequence he recognizes that life, in a truly remarkable variety of manifestations, can be found in every locality where systems with potential chemical energy are present. In view of the dynamic nature of hydro- and lithosphere this implies that in special locations populations flourish and decay, to be superseded in the same or adjoining areas by yet different ones.

After the recognition of the remarkable diversity in microbial metab-

olism had gradually gained ground, there were many who felt attracted to a more profound study of these phenomena. I shall restrict myself to indicate a few main lines along which our perception has increased as a result of these investigations.

In the first place, the various and divergent energy-providing processes have been subjected to a closer analysis. The respiratory process, during which some carbohydrate reacts with oxygen and is hereby converted into carbon dioxide and water, was one of the first processes into which a more profound insight was gained. In view of the fact that, at the low temperatures prevailing in our biosphere, sugars do not react at all with oxygen, it is highly remarkable that this process occurs quite intensively in the interior of many living cells. Pertinent investigations have shown that the glucose molecule undergoes a long series of transformations, the most fundamental among which are undoubtedly those reactions in which hydrogen atoms are removed from one of the intermediate products, and, under the influence of a large number of interposed catalysts, are transferred with the ultimate formation of a compound that can spontaneously react with free oxygen. In other words, the intermediate products formed from the sugar function as *donators* of hydrogen atoms, for which ultimately the oxygen serves as *acceptor*.

Now the concept thus developed, *viz.*, that the most common type of respiration is essentially based on a catalytic transfer of hydrogen, has turned out to be the key to an understanding of the numerous other energy-providing processes. Soon a similar situation was also encountered in those cases where substances other than sugars serve as the respiratory substrates. These, too, appeared to function directly or indirectly as donators of hydrogen atoms that ultimately found their way to oxygen. Hence this means that a single fundamental principle lies at the root of the diversity of metabolic phenomena exhibited by respiring micro-organisms that is at first sight so confusing. In the course of time life has apparently adapted itself to quite a variety of hydrogen donators, thus enormously extending the range over which it can exist.

But even this was not the end of it. I have already mentioned that in the microbial world there also occur numberless types with mutually extremely divergent metabolic patterns that do not require free oxygen for their perpetuation, nay, whose development may even be in-

hibited by this gas. That, despite the external variety, the same principle again lies at the root of the metabolism of these so-called anaerobic organisms is immediately shown by those anaerobes which, in addition to an organic substrate, also require for their energy-yielding process some specific inorganic substance. This situation is found with bacteria known as nitrate-, sulphate-, and carbonate-reducing organisms. If now one examines the fate of these substances in the metabolic process it appears that, at least under suitable conditions, the nitrogen, sulphur, and carbon, respectively, of these salts can be recovered in their most highly reduced states, *viz.*, as ammonia, hydrogen sulphide, and methane. Thus the perfect analogy of these conversions and of the respiratory process with oxygen is immediately emphasized, because in the latter this element is equally converted into its most reduced state, water. But then the notion presents itself that in the energy-providing processes in question the specific inorganic substances have evidently adopted the rôle of oxygen as hydrogen acceptor. Earlier we have seen that as far as energy provision is concerned, life has adapted itself to a great variety of hydrogen donors; now we learn that oxygen by no means needs to be the exclusive hydrogen acceptor, but that certain living organisms have become adapted to the use of entirely different hydrogen acceptors.

Finally we shall consider those anaerobic energy-yielding processes in which only one single organic compound is transformed, processes that are known by the terms fermentation and putrefaction. In these cases the fermentable substrate appears to undergo a fission from which originate on the one hand molecules that can function as hydrogen donors, and on the other hand molecules that can serve as hydrogen acceptors. Consequently the principle of catalytic hydrogen transfer as the basis of energy provision is encountered in these cases as well.

So far I have discussed only one aspect of metabolism, however; no less important are those metabolic processes in which particular food components are converted into cell materials, necessary for the repair of wear and tear, and for multiplication of the organism. Here, too, one is immediately struck by the great variety in requirements that different groups of microbes display with respect to their nutrition. Some amongst them are no less exacting than the human organism; in sharp contrast to these are types, such as the hydrogen bacteria,

that can multiply in a simple medium composed of hydrogen, oxygen, carbon dioxide, and minute amounts of some inorganic salts. By far the majority of micro-organisms appears to represent types that form a virtually continuous transition between these extremes. This implies that various microbes exhibit diverse nutrient requirements for the synthesis of proteins with which, as we know, life is associated. Whether, as in the case of the hydrogen bacteria, the protein is manufactured from the above-mentioned simple ingredients, or, as in the lactic acid bacteria, only from a complex mixture of protein degradation products, nevertheless the protein appears in every case to be composed of practically the same building blocks, *viz.*, the twenty-odd amino acids that have also been found in the proteins of man, animal, and plant. Nowadays this result tends to be accepted as self-evident; yet, in view of the variety of processes that have been employed by various organisms for the synthesis of their proteins, this could certainly not have been predicted. Once again we recognize in these facts a most exalting demonstration of life's unity.

And there is even more. Occasionally the nutrient requirements of micro-organisms bear a special character; it was, for example, ascertained that the addition of trace amounts of some cell extract to an initially inadequate medium sufficed to permit the growth of an organism. Continued investigations, in which our fellow-member, Kögl, has had an important share, have shown that the special activity of the cell extract was due to the presence of one or more organic substances of relatively simple composition, and which in part govern the life or death of an organism in astoundingly small concentrations. As is known, a fully comparable situation is encountered in the nutrition of man and animals, where extremely low concentrations of substances known as vitamins likewise determine the well-being of the organism. But the most important point is the fact that in many cases the identity of certain microbial growth factors with known vitamins has been established.

It is doubtful whether the significance of this discovery has been fully appreciated. It is true that physiologists have gratefully taken advantage of the opportunity to use micro-organisms as an aid in the detection and quantitative estimation of the vitamins they were studying. The essential point is, however, the great general biological significance of the concept that the nutrient deficiencies of man and

animals do not represent isolated phenomena but are, on the contrary, also encountered among quite remote groups of organisms. Is it not edifying that immeasurably small amounts of vitamin B-12, for example, which protects man from the horrors of pernicious anemia, may, under certain conditions, also determine the well-being of a small flagellate?

In the course of extended studies concerning the manner in which vitamins and growth factors exert their favourable effect on the behaviour of such diverse organisms it has become clear that they are partly components of fundamentally important catalytic systems that operate in cellular metabolism. Understandably, this has raised the question how cells can get along and perform their normal vital functions without growth factors in their food. Almost imperceptibly this has led to an important new notion, to the effect that those cells that do not require growth factors have certainly not renounced the use of those convenient tools, but differ from the growth factor-requiring ones in that they are capable of synthesizing the agencies in question themselves.

The fact that a sulphur bacterium that has developed at the expense of such simple inorganic substances as sulphur, oxygen, and a few salts appears to be a veritable storehouse of numerous vitamins that are indispensable for human nutrition provides food for thought. Given the absolute dependence of human health on a continuous supply of these vitamins, there is every reason for paying homage to the puny microbe that needs these substances just as much, but manages to manufacture them practically from the elements. Consequently the unity in the material basis for life does not express itself only in the comparable composition of the proteins of all living organisms, but obviously the same catalytic systems are employed by green plants, animals, and microbes, for the performance of their normal functions.

The far-reaching similarity in material composition of individual living entities, together with the earlier discussed unity in the multifarious energy-yielding processes, evidently provide a not-to-be-under-rated support for the evolutionary theory which, in its broadest formulation, holds that life is one and continuous, both in space and in time. I must refrain from discussing the developments that have led to the evolutionary theory; a few remarks may here be launched.

In the first place, the human mind cannot be satisfied by the alternative of accepting innumerable *de novo* creations; numerous factual observations can hardly be harmonized with this idea. Furthermore, there is the unequivocal testimony of the fossil remains of forms of life that have populated the earth during consecutive epochs; in numerous instances these reflect an unmistakable relationship between the inhabitants of successive geological periods.

However, the concept of evolution receives its strongest support from the results of modern genetics studies. It was in this very hall that, in 1900, Hugo de Vries expounded his ideas concerning the saltatory hereditary changes for which he coined the term mutations. A month later, Beijerinck discussed in connexion herewith the variability of micro-organisms. In a footnote to the corresponding publication Beijerinck declared his acceptance of the principle of mutation; some ten years later he furnished an impressive contribution to our knowledge of mutational phenomena in microbes. But only during recent years has the high frequency with which mutations occur in the microbial world been clearly illustrated, and this has cast a bright light on its significance as a general characteristic of life. Even though anything but agreement has been reached with respect to the forces that govern evolution, this situation makes it nonetheless understandable that many investigators proclaim the principle that reduces evolution to a random mutation, coupled with selective action of the environment.

We must keep in mind, however, that the fossil documentation of pre-historic life is practically limited to the last 500 million years of the earth's history. In a sense this is rather a short episode, at least if one takes into account the fact that until recently the age of our planet was estimated at more than 3,000 million years, while the most recent conclusions indicate that it may even be 4-5,000 million years.

If we accept the paleontologist's view that many of the phyla distinguishable among the present-day animals have existed during the above-mentioned span of 500 million years, *i.e.*, during the era beginning with what the geologists have termed the *Cambrian*, the evolutionist cannot escape the inference that life must have originated in still earlier times. The lack of fossils in the *Pre-cambrian* must then be ascribed to the fact that the living organisms that then existed were so small and fragile that, once enclosed in the sedimentary strata, they

failed as a rule to leave lasting impressions behind. I said as a rule, because during the past few years a number of exceptions have come to light. Not without a thrill was I confronted, during last year's visit at Harvard University, with the fossil microbes discovered by Tyler and Barghoorn, and whose age has been estimated by these scientists at approximately 2,000,000,000 years. The surprisingly intact forms leave no doubt that this oldest thus far known relict of life already possessed a cellular structure.

Modern cytological examination has indubitably shown that even the single cell represents a highly organized unit, in which easily recognizable structural elements, such as the nucleus, protoplasm, mitochondria, and many others, cooperate in a remarkably harmonious manner.

At first sight one might therefore be inclined to conclude that an evolution of life on earth can have begun only after such a complex living unit had somehow found its way to a sufficiently cool terrestrial surface. But for the true-blooded evolutionist it is tempting to conclude that life will have passed through a pre-cellular phase in which the vital characteristics of the various units were reduced to the bare essentials. And this almost selfevidently leads to the question in how far it is conceivable that at some time a spontaneous development may have yielded such primitive expressions of life on an initially lifeless earth. This idea, implying a continuity of living and inanimate matter, seems so daring that one might be inclined to dismiss it instantly; nevertheless, several prominent scientists – I shall but mention the great geneticist, H. J. Muller – have not shrunk from it. They derive their support, at least to a degree that cannot be underrated, from the consideration that for the reconstruction of this developmental process they may avail themselves of a time span of no less than 1,000–2,000 million years. And the proud edifice constructed by present-day geology is, so to speak, one concerted demonstration of the fact that the application of a time-coordinate measured in millions of years renders otherwise unimaginable matters intelligible to the human mind.

Naturally, a test of the projected problem is beset with great difficulties. Given the absence of visually observable testimonials of life during the earliest period of the earth's history, one can only try to infer what physical and chemical conditions were realized during this epoch, and contemplate the question in how far phenomena may be

postulated to have occurred such that they represent a continuity with the essential expressions of life as are known to us to-day. An adequate solution of this problem obviously requires the cooperation of exponents of many divergent fields of science.

I shall now attempt to draw a sketch of ideas that have been developed in this connexion, but before doing so I wish to mention that the outlines of the picture have been developed by many scientists amongst whom I shall name only such outstanding contributors as Haldane, Oparin, Horowitz, Bernal, Urey, and H. J. Muller. I, for my part, cannot refrain from including some concepts derived from studies of microbial metabolism.

Let us begin by consulting the astronomers who recently have advocated the view that the earth owes its origin to an agglomeration of small solid particles, previously formed in the primeval nebula from which our planetary system was born. Such an agglomeration must have led to a considerable increase in temperature, in consequence of which the earth initially must have appeared as a blazing liquid sphere. In his recent interesting discussion of the origin of our atmosphere our fellow-member, Berlage, has concluded that the earth's mass is too small to have prevented, at the existing high temperatures, the escape into interstellar space of an important part of the gases that at first surrounded it. Consequently an atmosphere, later developing after a period during which the earth became progressively cooler, must be of secondary origin, and have arisen from gases emanating from the earth's crust itself. However, the composition of this secondary atmosphere must have been quite different from that of our present atmosphere.

Especially the geochemist, Urey, has advanced forceful arguments in favour of the view that the most important components were hydrogen, methane, water, ammonia, and hydrogen sulphide. That is to say that there occurred in this atmosphere, besides hydrogen, the elements carbon, oxygen, nitrogen, and sulphur in their most completely hydrogenated state. During prolonged cooling a large part of the water must have condensed, and thus occasioned the coming into existence of the oceans that cover the greater part of the earth.

Gradually the earth's surface reached a temperature range within which life may have made its appearance upon the earth. It is, however, imperative to reject the idea that such life would have exhibited

any similarity with some of the recent forms of life, first of all because oxygen, indispensable for most of the latter, was lacking. But the earlier mentioned anaerobic metabolic types cannot have existed either because the entire environment was obviously in a greatly reduced state. Although there was, therefore, certainly no dearth of hydrogen donors, the equally necessary hydrogen acceptors cannot be found in this environment.

The principal argument against the notion that the first living entities would primarily depend on some simple inorganic chemical system is, however, the consideration that the conversion of its components into organic matter, though in principle conceivable, assumes nevertheless the presence of a primary living entity. And, in view of the earlier demonstrated great complexity of even the smallest autotrophic forms of life, a spontaneous appearance of a first living entity in this primitive environment is entirely inconceivable.

Haldane and Oparin have shown the way out of this dilemma by introducing what, with the wisdom of hindsight, now appears so obvious a notion, to wit that the origin of the first living organism must have been preceded by an accumulation of organic matter. Hence the question is whether there would be any reason for assuming that, during the developmental stage of the earth we are now considering, a strictly chemical formation and accumulation of organic matter may have occurred. The above-mentioned scientists have answered this question in the affirmative, and adduced strong arguments in its favour. Bernal in particular has emphasized that during this period the earth was subject to powerful ultraviolet solar irradiation of very small wavelength; it has been established that such radiation can exert profound chemical effects. At present we are protected from this strongly biocidal radiation by the ozone layer in the stratosphere; but on account of the absence of oxygen in the primitive atmosphere this screen was initially lacking.

Now it is entirely possible that this short-wavelength radiation, acting on a system composed of methane, ammonia, hydrogen, and water, may have led to the formation of organic compounds. The radiation would have caused a fission of water to hydrogen which escapes, and hydroxyl which would have promoted a partial oxidation of hydrogen donors, especially methane, and as a result of these reactions organic hydrogen acceptors became available. In this con-

nexion Urey has also pointed to the mighty electrical discharges that characterized this epoch, and it is certainly of interest to note that Miller shortly afterwards reported that under the influence of this agency amino acids are soon detectable in the above-mentioned system. If, furthermore, it is realized that these photo- and electrochemical processes must have proceeded for periods of many hundred million years, the conclusion seems almost irrefutable that gradually enormous quantities of organic matter must have accumulated in the primitive oceans. Moreover, as a result of the production of radicals by the above-mentioned agents it may safely be assumed that quite divergent types of organic substances must have originated through condensation reactions. Amongst these the building blocks of present-day cellular constituents must surely have been present. In the absence of life these compounds could persist in the aqueous medium, so that gradually the oceans became solutions of organic compounds.

It is this proto-solution that lends itself to making a spontaneous appearance of an utterly primitive form of life conceivable. In illustrating this I shall follow H. J. Muller's recently published discourse. First of all, this geneticist stresses the fact that the most essential components of life are the genes, the hereditary units that possess the power of multiplication under certain conditions, as revealed during every cell division. Chemically they can be characterized as nucleic acids, substances that are composed of numerous building blocks of, for the rest, a small number of types. The virtually unlimited diversity in arrangement of these building blocks determines the differences between individual nucleic acids, and consequently the specificity of the multifarious cells. In a sense we may therefore say that life, reduced to its ultimate essence can be viewed as a nucleic acid that attracts new building blocks, arranges these in accordance with its own pattern, and in this manner creates its physico-chemical image.

Bernal has pointed out that particular clays have the property of adsorbing very diverse organic molecules in a regular fashion, and also can function as catalysts of reactions that lead to a condensation of the adsorbed molecules. In this manner the first nucleic acid may have been generated. Under conditions that nowadays are still realized in every living cell, but have as yet been insufficiently elucidated, building blocks could have grouped themselves in regular order, ultimately causing the formation of a second molecule. This ordering in

the chaos of dissolved substances could then continue until the supply of one or more of the specific building blocks had become exhausted.

There are indications from cell physiology that the nucleic acids of the nucleus also give rise to the formation of the chemically somewhat different ribonucleic acids that are dispatched into the protoplasm surrounding the nucleus, where they function as catalysts for the synthesis of proteins. Although our understanding of the mechanism of these processes is still limited, it remains possible that, during the hundreds of millions of years that are available, local conditions have fortuitously been realized under which could be produced a system composed of desoxyribose nucleic acid – ribose nucleic acid – protein. Now the process of ordering the organic matter with the production of self-multiplying primeval living entities could proceed until the supply of some of the indispensable building blocks of the system had been depleted.

We have already seen that modern genetic research has convincingly shown the occurrence in microbial cells of spontaneous modifications, with a surprisingly high frequency, and referable to an alteration in the arrangement of the building blocks in the nucleic acids of the genes. On the other hand, investigations of the microbial mutations induced by irradiations have demonstrated that the various elementary reactions to which metabolism can be reduced are individually determined by specific genes. In this manner one can assume – and here I follow the line of reasoning developed by Horowitz – that an incidental mutation in a gene of a primeval living entity has caused this system to acquire the property of manufacturing the lacking building block from another kind of molecule with a closely corresponding composition. At this point metabolism proper made its entrance on the scene of life. Consecutive mutations, each one adding a new reaction step to the existing ones, thus caused the genesis of systems that became progressively more independent of the nature of organic substances present in the medium. Starting with a system that originally was heterotrophic with respect to every one of its building blocks, a situation that at present is still approximated by the obligatorily parasitic micro-organisms, there would gradually have arisen systems that became autotrophic with reference to an increasing number of building blocks. Ultimately this would have led to a completely autotrophic metabolism such as that which to-day we still encounter,

for example, in the sulphur bacteria. During this gradual development of metabolism to the degree of complexity that we find in present-day cells, more and more accessory systems must have become involved, and this must needs have gone hand in hand with a closer organisation of the cooperating components. Finally there would thus have appeared a complex that exhibits the essential characteristics of a cell.

Regardless of whether one is inclined to accept the existence of such a period in the evolutionary history of life, and which we may designate as pre-cellium, or prefers different concepts concerning the manner in which the living cell made its appearance on the earth, there will always be occasion for considering the possibility of an evolution of this first perfect living entity. In whatever manner, this cell must have found in the aqueous environments on the earth, *i.e.*, in the proto-solution, the conditions for its development. This raises the question in how far mutations and the continuous changes in the external environment resulting from metabolic activities may have led to the appearance of novel modes of life that could exploit new areas.

It is exactly the earlier discussed fundamental unity in composition of the most divergent recent cells that lends strong support to this possibility. It will be necessary, however, to remember that a multiplication of living entities, whether precellular or cellular, can be postulated only if this process can be coupled with energy-yielding processes. Initially the proto-solution contained an abundant supply of organic hydrogen donators and acceptors. As the anaerobic mode of life unfolded more and more, the supplementation of organic matter, produced by the short-wavelength ultraviolet radiation in the upper layers of the oceans, must have lagged behind the utilization of these substances in the deeper layers.

The interplay between the organic donators and acceptors must eventually have led to the production of carbon dioxide and more or less completely hydrogenated acceptors. These, in turn, might have been used as donators, provided an adequate acceptor was simultaneously available. In this respect only carbon dioxide can ultimately be considered, and, as we know from the metabolism of the recent methane producing bacteria, it can indeed be converted into the fully hydrogenated form of carbon methane. Meanwhile, the production of carbon dioxide by the continued dehydrogenation of the

organic donators must obviously have outweighed the amount consumed. And because the methane escaped into the atmosphere, the end result must have been a decrease in the concentration of organic donators and an increase in that of carbon dioxide.

As additional hydrogen donator the medium must initially also have contained hydrogen sulphide. But the system H_2S plus CO_2 does not in itself present any metabolic possibilities. At this stage there must, however, have occurred a startling event. At a certain moment the continually changing life must have acquired the ability to synthesize compounds that possess the property of not merely absorbing radiation in the visible and near-infrared region of the spectrum, but of doing so in a manner in which the energy of the absorbed quanta can be made subservient to the metabolic process. By virtue of this mechanism it became possible to hydrogenate carbon dioxide to cell material with hydrogen sulphide as hydrogen donator, and with the concomitant formation of sulphate as dehydrogenation product of the sulphide. Even to-day the sulphur purple bacteria bear witness to the efficacy of this remarkable mode of life.

With the exploitation of solar radiation as energy source, life, in its collective sense, liberated itself at once from its dependence on an external supply of organic matter. For the radiant energy was, so to speak, transformed into organic matter and acceptor, so that at the same time the conditions were created for the existence of sulphate reducing bacteria which could reconvert the system in question into the raw materials for photosynthetic life. On this basis anaerobic life created the possibility for an autonomous perpetuation *ad infinitum*, at least as long as the influx of solar energy remained assured.

Yet the enormous significance of this development is overshadowed by the next stage in biochemical evolution. In a living entity that based its existence on the system, radiant energy-hydrogen sulphide-carbon dioxide, random mutations led to new forms of life capable of substituting water for hydrogen sulphide as hydrogen donator. This entailed dramatic consequences, firstly because of the virtually unlimited availability of the new donator, water. As a result of the elimination of the need for other donators the potential area of life's existence immediately became enormously expanded. But of still farther-reaching significance was the circumstance that oxygen was produced as dehydrogenation product of the water. This gas, escaping into the

atmosphere, gave rise to the appearance of the ozone layer, screening out the biocidal short-wavelength ultraviolet solar radiation, and thus for the first time offering life the opportunity to leave the hydrosphere and to invade the mainland. The first terrestrial inhabitants will also have exhibited a photo-autotrophic metabolism, and consequently belonged to the green plant kingdom. But the most beneficial effect of the birth of free oxygen on earth was undoubtedly that this gas lends itself pre-eminently to serve in its turn as hydrogen acceptor. The oxygen, and the organic matter that becomes available as hydrogen donor upon the death of photoautotrophic organisms, together constitute the system on which is based the aerobic mode of life. It must furthermore be realized that not until the gaseous hydrogen acceptor, oxygen, had appeared did the conquest of the atmosphere as a realm of life for the animal organism become possible.

Herewith I bring to an end my sketch of evolution; if one desires to acquire an idea of the manner in which aerobic life evolved from its inception to the present-day plant and animal kingdoms, one has to sit at the feet of the paleontologist. I have wished merely to indicate that a knowledge of microbial metabolism such as, even to-day, it presents itself to us in its surprising diversity, can unquestionably contribute to the acquisition of some insight into the first, and as yet still obscure, phases of evolution.

My audience may pardon me this effusion. I realize that the discussion concerning the evolution of life on earth must have left many of you with the impression that I was spinning a fairy tale. I shall not excuse myself by referring to the fact that the eminent *savant* who addressed the meeting of the combined sections a year ago also requested your attention for a fairy tale; but I should like to remark that, if a fairy tale be defined as a story that contains elements not immediately acceptable to human reason, a survey of results of investigations in very diverse branches of natural science must almost always create the impression of a fairy tale in the outsider. Let me illustrate this with an example.

I shall choose the physicist's concept of the structure of matter. The layman, examining for instance a sugar crystal, will perceive something altogether static, and conclude to a fully homogeneous composition. Nevertheless the physicist, with a pitying expression, will explain to

him that this substance is composed of an immense number of mutually identical units, all of which are in active motion; furthermore, that each of these molecules is again composed of 45 smaller units, comprising three different kinds, the so-called atoms, that are also subject to various movements inside the molecular combination. And if one considers each one of these atoms, the physicist will tell you that these represent a small body, referred to as the nucleus, surrounded at relatively great distances by infinitesimally small particles moving around the nucleus at enormous speed. And even the nucleus is far from homogeneous; various components, in part still incompletely characterized, appear to exist therein, and to carry out all sorts of curious motions.

This, then, is the physicist's fairy tale; nevertheless, the atomic bomb is an eloquent testimony to the significance of this picture. But I might, and with equal justification, have regaled you with one of the many fairy tales of the geologist, or with the most beautiful fairy tale of all, the astronomer's concept of the structure of the universe. Time does not permit this; it is, however, certain that the capacity of the layman to imagine these things is scarcely adequate.

Now I realize full well that many of my colleagues will be severely shocked when they find their most profound concepts designated as fairy tales. In this context I should like to remark that these concepts have not developed overnight; many others have preceded the current version, and it is far from certain that the latter will not be succeeded by still different ones. It is therefore possible to speak only of various degrees of certainty as approximations to reality. Hence I want to express the hope that you will also grant the microbiologist his fairy tale; for such tales derive their value from their nature as working hypotheses, and as such they inspire the investigations that eventually will increase man's comprehension of nature.

Meanwhile, it is high time to return to my point of departure, and to attempt a summary of the microbiologist's concept of life. He does not recognize merely the virtual ubiquity of life, but also the abundant significance of the invisible part of life that exists, as it were, underground; furthermore, the complete mutual dependence of both the higher and the lower forms of life; and, above all, the surprising diversity in the manner in which different living beings have succeeded

in making the various chemical systems found on earth serve their existence and multiplication. Yet all of this is dominated by the recognition of the fundamental unity so unmistakably displayed by the diversity of living forms; a unity that cannot otherwise be apprehended than on the basis of a common origin.

Thus the microbiologist is destined to acknowledge the potentialities that enabled life to flourish during an early phase of the earth's history, when there was as yet no question of green plant metabolism, based on radiant energy, nor of present-day animal metabolism which, after all, depends on oxygen produced by the plants. Hence, in the mind of the microbiologist there can hardly be room for doubt as to the validity of the thesis that, in its most primitive manifestations, life must have been anaerobic; and he will also be inclined towards the conclusion that it must have passed through a pre-cellular stage. This idea finally culminates in H. J. Muller's recent phrase that, seen from a cosmological viewpoint, 'the totality of life is merely a fancy kind of rust afflicting the surfaces of certain lukewarm minor planets'.

To many among my audience this discourse will have been a sore trial, especially because my analysis seems to encroach upon the reverence with which man naturally tends to view the mystery of life. Let me therefore emphatically state that the microbiologist, too, is fully cognizant of this mystery which, at certain times, is irresistibly forced upon him, for example when, bent over his microscope, he watches the playful movements of his single-celled objects. And he, no less than any other person, is receptive to the impressions of beauty and harmony that a contemplation of the infinite variety of the more highly organized living beings is apt to evoke.

But the desire to establish a closer acquaintance with the multifarious forms, and to reach a more profound comprehension of the phenomena of life, these are far from being desecrations. In this context I may refer to the thorough essay that our fellow-member, Dijksterhuis, recently devoted to the subject, 'Mathematics, natural science, and technology as cultural elements', and particularly to his remark that, from a Christian standpoint, nature is a revelation of God, and hence the study of nature a Christian's duty.

If, therefore, I have not immediately rejected the idea that some day we may come to conclude that there is continuity from living to

inanimate matter, then one should realize that this implies no more than that these two forms of matter were not created separately, but that one act of creation comprises all. The prime mystery remains; the magnificence of the creation is thereby not assaulted; quite the contrary.

We must also consider the following proposition. The biologist who, now and then, examines effervescent life in astonishment, sometimes mixed with aversion or even fear, will be conscious of the fact that he observes merely a momentary stage in life's evolution, and that the objects studied may be characterized as the by-products of an evolution that has also led to the appearance of that organism on whom has been conferred the grace of the spirit and the soul. With the appearance of man the collective principle of life did not only undergo a radical alteration; it also implied a new element in evolution. Prior to that moment life developed according to the 'trial and error' principle of a blind mutation; the road traversed was, so to speak, strewn with the remnants of mutations harbouring lethal factors, and of those that had fallen prey to the selective action of the environment. The advent of man brought about a change because indubitably there are forces hidden in the human mind that participate in determining the course of evolution.

On the one hand this can be made tangible, as it were, by saying that we have already progressed so far that an utter destruction of humanity, perhaps even of all life on earth, is now within the reach of human possibilities. But opposed to this is another aspect. The acquired insight into the laws of heredity enables man to re-create, so to speak, the forms of life by which he is surrounded, and by conscious selection preferentially to promote particular forms. The commandment, 'have dominion over the fish of the sea, and over the fowl of the air, and over everything that moveth upon the earth', acquires a new meaning in the light of present-day biological science.

And as far as man himself is concerned, already for a long time it has not been medical science only, but also mental factors, such as love and compassion, that have called a halt to evolutionary forces of a blind and decimating kind. Who dares predict to what a further unfolding of the human mind and spirit may yet lead?

Heavy is the responsibility that rests on mankind; may we succeed in finding the right way.

PART THREE

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1936

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- 1947-1954
President of the Koninklijke Nederlandse Akademie van Wetenschappen
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Honorary Member of the Society for General Microbiology, Great Britain
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ARTICLES DEDICATED TO A. J. KLUYVER AND HIS WORK

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IN MEMORIAM A. J. KLUYVER

HET plotseling overlijden van de grote geleerde, die Albert Jan Kluyver is geweest, heeft in ruimer kring dan die der beoefenaren van de biologie, wezenlijke droefheid gewekt. Zijn heengaan treft de samenleving der Nederlandse wetenschap als een zware slag en zal ver buiten onze grenzen met dezelfde verslagenheid worden vernomen.

In ruime mate is Kluyver bij zijn leven eer bewezen, getuigend van waardering voor zijn wetenschappelijke arbeid, in de vorm van ere-doctoraten en andere onderscheidingen, hem in binnen- en buitenland verleend. Doch daarnaast moet hij meermalen getroffen zijn geweest door hetgeen hem toesprak uit brief en oogopslag van de velen, die hem als onvervangbaar geschenk hun wetenschappelijke vorming dankten.

Hijzelf wekte bewondering door het resultaat van zijn studiën, zijn toewijding als geleerde en docent, de zekerheid van zijn weten en de onfeilbaarheid van zijn geheugen, door de wakkere en toch beschouwelijke geestigheid van zijn betoogtrant, bovenal door zijn warme bereidheid tot hulp voor ieder. En daarnaast verwondering dat in één mensenleven het bereiken mogelijk bleek van wat hij inderdaad volbracht. 'Pour le Professeur Kluyver, point d'heures de travail, point d'heures de repos, il est toujours occupé de son oeuvre', schreef een Franse medewerkster in 1936.

Van deze ononderbroken werkzaamheid moge thans in de beschouwing van enkele facetten getracht worden iets op te roepen van Kluyver's visie en levens-taak.

Toen in 1921 de jonge A. J. Kluyver het Laboratorium voor Microbiologie der Technische Hogeschool als hoogleraar binnentrad, was niemand meer dan hijzelf er van overtuigd dat het hier een crediet-benoeming betrof. Zijn opleiding immers, bij G. van Iterson Jr., was zeker niet specifiek op de microbiologie gericht geweest. Evenmin verrieden zijn maatschappelijke functies, bekleed tussen het afsluiten van zijn dissertatie en zijn ambtsaanvaarding te Delft, de toekomstige microbioloog, hoezeer ook gevarieerd en wetenschappelijk getint.

Terstond realiseerde Kluyver zich de moeilijkheid van zijn taak: de eminente en door hem zeer bewonderde geleerde Beijerinck op te volgen en een laboratorium van wereldnaam op het bereikte niveau te handhaven.

Met een sterk gevoel voor ordening en systematische opbouw, die beide ken-

merkend zouden blijken voor al zijn verdere onderzoeken, bestudeerde Kluyver de verscheidenheid in de ééncellige micro-organismen en wist hun levensverrichtingen, zich uitende in omzettingen van chemische stoffen, vast te leggen. Doch reeds in deze eerste studies uit zijn laboratorium bleek, dat hij – ondanks de hem kennelijk boeiende verscheidenheid – naastig zocht naar een bindend element in al deze verschijnselen.

Zijn grote gave tot combineren, zijn overtuigd streven naar het herkennen van het essentiële, kwamen pas duidelijk aan het licht, toen er in de wetenschap van die dagen een strijd ontbrandde over de wijze, waarop de zuurstofoverdracht bij de ademhaling verloopt. In de gedachte van Kluyver waren de verschillen, waargenomen bij de stofwisseling der micro-organismen, terug te brengen tot een gevarieerde aaneenschakeling van eenvoudige, stapsgewijze processen, die tot slechts enkele prototypen zouden zijn te beperken. Met zijn medewerker Donker en tegelijkertijd met de Nobelprijswinnaar Szent-Györgyi, doch geheel onafhankelijk van deze, formuleerde hij het grondpatroon van zijn visie op de microbiologie. Zijn 'Eenheid in de biochemie' bracht met één slag ordening in de verwarrende hoeveelheid materiaal.

Het is nu opmerkelijk dat zijn conclusies niet slechts breder en meer generaliserend waren dan die van Szent-Györgyi, doch dat hij tevens reeds in deze tijd ten volle de grote betekenis van de studie der micro-organismen voor de fysiologie in het algemeen onderkende.

Indien inderdaad zijn werkhypothese – een essentiële eenheid in de stofwisseling van alle levensvormen – juist zou blijken, konden door experimenteren met de zo hanteerbare micro-organismen, conclusies van algemene strekking worden verkregen.

In de jaren, volgende op deze publikatie, werkte hij doelbewust deze hypothese uit. Het resultaat van een stelselmatig onderzoek van bacteriëngroepen met zeer verschillende stofwisselingstypen, neergelegd in een reeks van proefschriften, leidde op zijn gebied tot een volledige bevestiging van de hypothese. Het moet hem voorts een grote voldoening zijn geweest te bemerken, dat zijn ideeën ook buiten zijn vakgebied ingang vonden en bevruchtend werkten op onderzoeken over de stofwisseling van hogere organismen.

In het jaar 1930 bereikte Kluyver de uitnodiging een serie voordrachten te houden aan the University of London. In deze lezingen bracht hij opnieuw het thema van de eenheid naar voren en formuleerde hij de vergelijkende biochemie in haar doelstellingen. Hij aarzelde niet uit te spreken dat de vergelijkende biochemie, ofschoon op dat moment nog weinig ontwikkeld, in de toekomst dezelfde betekenis voor de biochemie zou verkrijgen, die de vergelijkende anatomie reeds voor de anatomie bezat.

Op grond van de onderzoeken der laatste decennia op het gebied der stofwisseling van microben en van planten- en dierweefsels, is zijn conceptie

van de eenheid in de levende natuur volkomen gewettigd gebleken. Haast vanzelfsprekend worden thans bij de bestudering van de meestuittenlopende fysiologische processen, weefsels van plant en dier in toenemende mate, en met succes, vervangen door de experimenteel zo toegankelijke microben. Het is stellig opmerkelijk dat zelfs op het terrein der genetica thans schimmels, gisten, bacteriën en bacteriofagen met stijgend resultaat de strijd aanbinden met de orthodoxe studie-objecten der genetici als teunisbloem, maïs en banaanylieg.

Men zou Kluyver te kort doen door hem alleen als geleerde te zien. Evenals hij een algemene lijn trachtte te ontdekken in de levensverschijnselen, – zo streefde hij ook naar een inzicht in de essentialia der fabriekmatige productie van chemische stoffen met behulp van micro-organismen. Het kan dan ook niet verwonderen dat hij én als geleerde én als technicus een omstreeks 1930 algemeen geldende fabrikage-methode met behulp van schimmels, fundamenteel wijzigde. Hierbij slaagde hij er in, met behulp van zijn medewerker Perquin, geheel nieuwe wegen aan te geven, die thans, vijf en twintig jaar later, zowel bij het wetenschappelijk onderzoek als in de industrie, algemeen ingang hebben gevonden.

Het lijdt geen twijfel dat de tegenwoordige wereldproductie van antibiotica, zonder de bijdrage der 'submerged culture', zoals die in Kluyver's laboratorium was uitgewerkt, niet mogelijk geweest zou zijn.

Naast deze aspecten verwaarloosde Kluyver ook de meer praktische vraagstukken der toegepaste microbiologie niet. Zo trok bij voorbeeld de margarine-industrie profijt van zijn onderzoekingen over het boter-aroma en een studie van bepaalde, voor de conservenindustrie schadelijke micro-organismen leidde tot praktische richtlijnen voor de bedrijven in kwestie.

Sterk zal voor allen, die Kluyver gekend hebben, naast de geleerde en de technicus, de docent blijven leven. Uit de veelheid van dikwijls tegenstrijdige literatuurgegevens verstond hij het een boeiende synthese te scheppen. Zijn periodieke overzichten van de stand der microbiologie en biochemie werkten stimulerend op de ontwikkeling dezer vakken, terwijl zij tevens bijdroegen tot een voortdurende verjonging van het door hem gedoceerde materiaal. Trouwens, ook in deze samenvattingen wist hij door opmerkelijke formuleringen de te verwachten gang van zijn wetenschap te schetsen. Ongetwijfeld moeten zijn toehoorders in Helsinki – vóór de Tweede Wereldoorlog – getroffen zijn geweest, toen hij voorspelde dat naast de groene plant, ook het zoogdier koolzuur zou blijken te assimileren. Studies met radio-actieve isotopen, in de daarop volgende jaren in Amerika uitgevoerd, stelden dit feit onomstotelijk vast.

Het is een gelukkige omstandigheid dat Kluyver nog zo kort geleden (in 1954), bereid werd gevonden – evenals zovele malen elders – aan the Harvard University tezamen met zijn leerling Van Niel een serie colleges te geven. Door het in druk verschijnen van deze lezingen is een belangrijk stuk van de visie

der door Beijerinck geschapen en door Kluyver en Van Niel uitgebouwde 'Delftse School' vastgelegd.

Zo werden in de internationale wetenschappelijke wereld Kluyver's opbouwende qualiteiten onderkend en ook in tal van organisaties ingeschakeld. Vele onderzoekers uit de gehele wereld richtten hun schreden naar de Nieuwe-laan, om hun werk aan zijn oordeel te toetsen of zijn advies te vernemen.

Kluyver was met grote dankbaarheid vervuld voor de wijze, waarop het hem gegeven was zijn vak te beoefenen en wellicht schuilt daarin mede de kracht die hem ten dienste stond.

Onvoldoende sprak uit het bovenstaande de invloed van Kluyver's persoonlijkheid op allen, die met hem in aanraking kwamen. Zij scherpe blauwe ogen waren, naast zijn proefobjecten, toch het liefst gericht op levende mensen, bovenal de jongeren, naar wie zijn belangstelling met onverborgten voorkeur uitging.

Velen zullen in deze dagen denken aan de uren, met soms benauwende vrijgevigheid hen geschonken in het ruime en toch zo besloten hoogleraarskabinet, op de grens tussen het Laboratorium en de oude ambtswoning, waar mede een onzienlijk contact gedurig merkbaar bleef. Het heengaan van haar, die met zuivere intuïtie voor zijn mogelijkheden, hem de gelegenheid schonk vrijwel al zijn tijd aan de wetenschap te wijden en haar man daarnaast ook actief behulpzaam wist te zijn, gaf zijn laatste jaren een verstilling, die niettemin aan deze eenzaamheid een glans van mildheid verleende.

Kluyver zal gemist worden in de Delftse hogeschoolgemeenschap, in de engere kring van vertrouwde medewerkers bij de gestadige arbeid van alle dag in zijn Laboratorium. In de Koninklijke Akademie mag zijn stem, met gezag en toch elke begrenzing onderkend, niet meer klinken, noch in de vergaderruimten, waar zijn bemiddelend woord trivialiteit tot niveau en kleine twist tot relatief geschil verhief.

Even sterk als zijn werk generaties durend zal boeien en telkens opnieuw slechts kan getuigen van de luister van zijn geest, zal wat de mens Kluyver voor de zijnen was, in harde arbeid en genegen begripen, blijvend toeven in hun herinnering als een warmend licht.

APOLOGY

THE plans for this book originated in an *ad hoc* committee, made up of persons who in some manner or another had been associated with Kluyver. Most of its members were pupils: they are: Miss Dr. M. P. Löhnis, Dr. L. E. den Dooren de Jong, Dr. B. Elema, Mr. K. W. H. Leeftang, Chem. E., Prof. Dr. T. Y. Kingma Boltjes, Mr. E. van Olden, M. Sc., Dr. L. H. C. Perquin, Dr. J. W. M. la Rivière, and Dr. W. Verhoeven. Following his appointment as Kluyver's successor, Prof. T. O. Wikén accepted an invitation to join the committee, which also included the author Mr. A. F. Kamp, LL.M. The editorial activities were taken charge of by Mr. Kamp, Dr. Verhoeven and Dr. la Rivière. Mrs. A. G. Waisvisz-Merens acted in an administrative capacity.

We, the committee members, keenly realize that the result of our labours could only have been attained by virtue of the aid and cooperation of many persons outside our own group. We are particularly indebted to Prof. Dr. C. B. van Niel, former associate, friend, and colleague, who has shouldered the task of the English translations, and who, through his intimate contacts, extending over more than thirty years, was fully conversant with Kluyver's work and thought. For this reason we appreciate that he has also been willing to outline the significance of Kluyver's contributions to science.

For his sketch of the early years, which ushers in the biographical memoir, we extend our thanks to Dr. W. E. van Wijk, a close friend of the pre-college days; we are obligated to Prof. Dr. A. van Rossem for the extensive and lively remembrances he has contributed, especially those pertaining to the years Kluyver spent in the Netherlands' East Indies, a phase with which Van Rossem was thoroughly familiar. Prof. Dr. E. C. Wassink, an initial member of the 'Biophysical Group' in Utrecht, has kindly supplied information concerning Kluyver's participation in the activities of this group. For the rest, the memoir comprises an edited version of the individual contributions of those committee members who are pupils of Kluyver. We consider the con-

cluding, contemplating part 'Kluyver as seen by his pupils' by Dr. W. J. M. la Rivière, since 1952 a co-worker of the laboratory, to be a happy addition to the knowledge of Kluyver's personality, especially because of its comprising qualities.

We express our appreciation to Prof. Dr. O. Bottema, Rector Magnificus of the Technological University, for granting premission to include the funeral oration he delivered at Westerveld, and to Prof. Dr. A. van Rossem for the version of his speech on that occasion.

It appeared desirable to confine the republication of Kluyver's *oeuvre* as much as possible to papers of which he was the sole author. Therefore only two articles have been included that were written in collaboration with associates [1926; 1936]. Though it would have been attractive to reprint also Kluyver's Doctor's dissertation, we were dissuaded from doing so, if for no other reason than its very size. Hence the 'Selected Papers' cover exclusively the span of his professorate. We have striven to make a selection such that it fairly covers this entire period.

Along with the inaugural address [1922], the lecture that represents a first reconnaissance [1924], and the publication [1926] that contains an outline of the impressive research program constitute a logical introduction. The 1931 and 1936 papers portray the search for an approach that would permit the fundamental unity in the diversity of microorganisms to find expression also in the field of systematics. The Helsinki lecture [1939] reveals Kluyver's early interest in carbon dioxide assimilation by microbes devoid of chlorophyll. The talent for expounding both a broad and a narrow subject, such as the evolution of microbiological science or a particular problem of more transitory significance, is clearly evident from the 1947 and 1952 papers.

For readers not trained in microbiology the inclusion of the 1937, 1949, and 1955 lectures, in English translations, affords an opportunity to acquaint themselves with Kluyver's approaches and concepts. At the same time these papers amply evince Kluyver's wide range of interests.

The scientific publication of 1953 shows Kluyver's undiminished interest in the problems concerned with hydrogen transfer. The other contribution of the same year divulges something of his ideas on the relationship between the scientist and his social environment.

As Kluyver would have insisted, we have endeavoured to spare no efforts on the composition and appearance of this book. With much understanding of the nature of our undertaking, Mrs. G. M. Pot-Van Regteren Altena in Schoorl arranged the design. We wish to thank Paul Huf for the frontispiece and for photograph 22, and particularly for the suggestions for the lay out of the photographic section. Publication and printing were in the hands of the North-Holland Publishing Co., Amsterdam, and of the printing establishment of Meinema, Delft, respectively. Kluyver used to speak highly of both these firms.

